The Use of Ipso-dihydrodiols Enzymatically Derived from Benzoic Acid in Enantioselective Synthesis. Appoaches to Total Synthesis of Vinca Alkaloids

David R. Adams

Department of Chemistry

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Faculty of Mathematics & Science, Brock University

St. Catharines, Ontario

ABSTRACT

The present studies describe our recent progress in target oriented synthesis of complex organic molecules from aromatic precursors. The latest synthetic approaches toward vinca alkaloids are described and include the construction of model substrates for the investigation into Diels-Alder, radical cascade, and tandem Michael addition reactions as possible routes to the family of alkaloids. Also described are the chemoenzymatic syntheses of the natural product (-)-idesolide and unnatural polyhydroxylated pyrrolidines generated from the biotransformation of benzoic acid with *Ralstonia eutropha* B9.

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[H]	hydrogenation
[O]	oxidation
[R]	reduction
2,2-DMP	2,2-dimethoxypropane
ACCN	1,1'-azobis(cyclohexane-1-carbonitrile)
ADP	adenosine diphosphate
AIBN	azobisisobutyronitrile
atm	atmospheres
ATP	adenosine triphosphate
AZADO	2-azaadamantane *N*-oxyl
BAIB	bis(acetoxy)iodobenzene
BC	before Christ
Bn	benzyl
Boc	*tert*-butoxy carbonyl
BPDO	biphenyl dioxygenase
BZDO	benzoate dioxygenase
Bz	benzoyl
*C. rose*us	*Cantharanthus roseus*
cAMP	cyclic adenosine monophosphate
CSO	camphorsulfonyl oxaziridine
d/l	dextrorotatory / levorotatory
d.r.	diastereomeric ratio
D4H	desacetoxyvindoline 4-hydroxylase

DAT	deacetylvindoline *O*-acetyltransferase
DHB	1,2-dihydro-1,2-dihydroxybenzoic acid
DIBAL-H	diisobutylaluminum hydride
DIPEA	diisopropyl ethylamine
DME	dimethoxyethane
DMF	dimethylformamide
DMAP	dimethylamino pyridine
DMP	Dess-Martin periodinane
DMSO	dimethylsulfoxide
e.e.	enantiomeric excess
e.r.	enantiomeric ratio
EPA	Environmental Protection Agency
G10H	geraniol-10-hydroxylase
glac	glacial
Glu	glucose
GSH	glutathione
HOMO	highest occupied molecular orbital
HPLC	high pressure liquid chromatography
HTOM	16-hydroxytabersonine-16-*O*-methyltransferase
Hunig's base	diisopropyl ethylamine
IBA	iodosobenzoic acid
IBX	2-iodoxybenzoic acid / *o*-iodoxybenzoic acid
IPP	isopentyl diphosphate
IR	infrared spectroscopy
KHMDS	potassium hexamethyldisilazide

LUMO	lowest unoccupied molecular orbital
MOM	methoxymethyl
MS	mass spectroscopy
MW	molecular weight
NDO	naphthalene dioxygenase
NMO	*N*-methylmorpholine
NMR	nuclear magnetic resonance
NMT	*N*-methyltransferase
OKR	oxidative kinetic resolution
Oxone	potassium peroxymonosulfate
PS-BAIB	polymer-supported bis(acetoxy)iodobenzene
Pd/C	palladium on charcoal
Pgp	P-glycoprotein
PLE	pig liver esterase
PNP	purine nucleoside phosphorylase
PPTS	pyridinium *p*-toluenesulfonate
p-TsOH	*p*-toluenesulfonic acid
py	pyridine
rt	room temperature
rac	racemic
SAM	*S*-adenosyl-L-methionine
SEMCl	2-(Trimethylsilyl)ethoxymethyl chloride
SET	single electron transfer
T16H	tabersoninie-16-hydroxylase
TBAF	tetrabutylammonium fluoride

TCCA	trichloroisocyanuric acid
TDO	toluene dioxygenase
TEMPO	(2,2,6,6-tetramethylpiperidin-1-yl)oxidanyl
Tf_2O	triflic anhydride
TFA	trifluoroacetic acid
TFAA	trifluoroacetic anhydride
TLC	thin layer chromatography
TMS	trimethylsilyl
TMSOTf	trimethylsilyl trifluoromethanesulfonate
TPP	tetraphenylporphyrin
TPAP	tetrapropylammonium perruthenate
Troc	2,2,2-Trichloroethyl carbonate
UV	ultraviolet
UV-Vis	ultraviolet – visible

1. Introduction

In 1975 Hendrickson stated that the "ideal synthesis" creates complex molecules in such a way that construction of the skeleton and functionality involves no intermediate transformations and leads directly to the target.[1] Since Hendrickson's rather systematic assessment for synthetic design many economy metrics have been designed to guide the practitioner toward an efficient synthesis. Three main classes of synthetic economies can be defined: step economy, atom economy, and redox economy. The ideas are simple and obvious from the names. Step economy implies that by minimization of the number of steps leads to an efficient synthesis in terms of both cost and time.[2] Strategic reaction sequences in the early development stages dictate the success in achieving a step-economical synthesis. This is the design space in which creativity and efficiency are manifested. Atom economy, a term first defined by Trost,[3] states that an efficient chemical reaction incorporates all of the atoms of the starting materials into the desired product. In other words, the percentage of the molecular weight (MW) of the starting materials should be present in the molecular weight of the desired product after several chemical transformations. However, catalysts, solvents, and reagentexcesses are not usually included in atom economy analyses. A good example of this metric is the Diels-Alder reaction (100% atom economy). Atom economy is arguably one of the major metrics considered in the chemical industries given the cost of raw materials, production and waste treatment. Redox economy is an ideal in which non-strategic redox manipulations are minimized and a synthetic target is approached in such a way that the oxidation states of intermediates should not fluctuate but increase or decrease steadily,[4] the consequence of which is also step economic.

The use of biocatalysis to generate a chiral pool of raw materials, synthons, or to efficiently transform a reaction center exemplifies many of the reaction metrics previously mentioned. Enzymes are capable of performing chemical transformations with extraordinarily high levels of stereo-, regio-, and chemoselectivity. The efficiency with which nature's catalysts can perform chemical transformations and the selectivity possessed is unmatched by modern chemical methods. For these reasons biocatalysis is being utilized to a greater extent and is now considered as a viable option when a synthetic route is being developed. Several academic groups have adopted the use of mutant organisms to access enantioenriched intermediates for use in synthesis.

Gibson's development of the mutant strain *P. putida* 39D, and of the engineered strain *E. coli* JM109 (pDTG601), allowed metabolizing haloarenes **1** to *cis*-dihydrodiols **2** by toluene dioxygenase (TDO). The process is high-yielding and stereo- and chemoselective. Metabolites derived from arenes have been used extensively for the synthesis of complex molecules, Figure1.

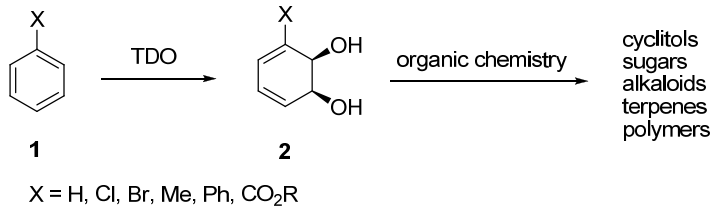


Figure 1. Metabolism of arenes by toluene dioxygenase.

In 1971, Reiner and coworkers described the use of a mutant microorganism *Ralstonia eutrophus* B9 that was capable of metabolizing of benzoic acid (**3**) into *ipso* substituted *cis*-dihydrodiol **4**, Figure 2, by the action of benzoate dioxygenase (BZDO). To date, metabolite **4** has not been used to the same extent as the TDO-derived metabolites. However, many research groups have recognized the utility of BZDO-

derived **4** for its unique substitution pattern, the quaternary center, and the fact that it is produced in enantioenriched form (> 95% ee). In this dissertion the utility of *cis*-dihydrodiol **4** in enantioselective synthesis will be described, in particular, the synthesis of natural product (-)-idesolide (**5**), the synthesis of iminosugar derivative **6**, and general strategies toward the synthesis of highly oxidized vinca alkaloids, such as vindoline (**7**).

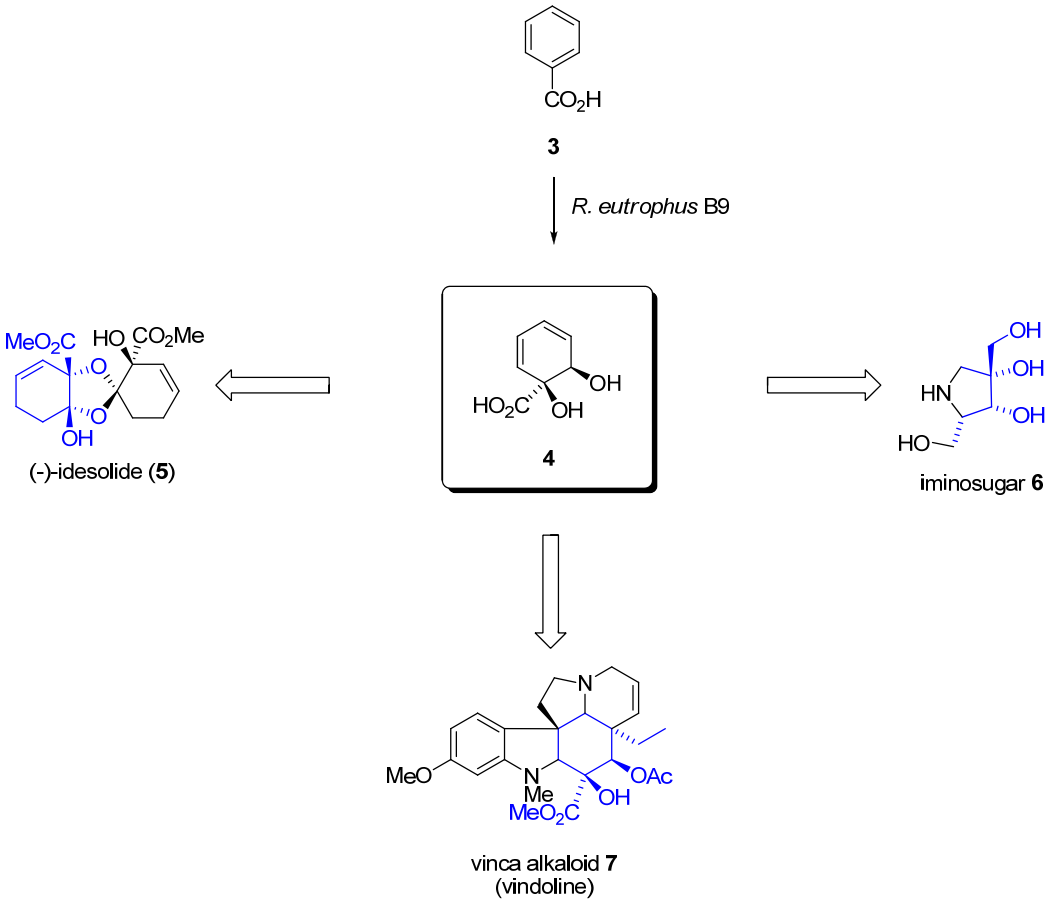


Figure 2. The projected use of *ipso* dihydrodiol **4** in synthesis.

Idesolide (**5**) contains unique structural architecture that has attracted the interest of organic chemists since its isolation in 2005.[5] Idesolide contains a spirocyclic motif that was accessible *via* the dimerization of monomer **8**. The resemblance of enzymatically derived dihydro diol **4** with that of idesolide led us to pursue the synthesis based on the

analysis below, Figure 3. The details of the synthesis and its comparison to the two other total syntheses will be discussed.

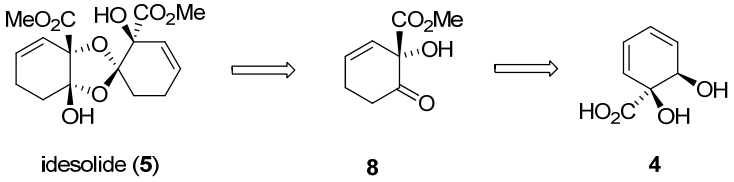


Figure 3. Idesolide approaches and syntheses.

Iminosugar derivatives are attractive targets in many medicinal chemistry programs and are known to be potent inhibitors of many enzymes, such as glycosyltransferases, glycosidases, and glycogen phosphorylases. Our approach to the synthesis of iminosugar **6** originates with metabolite **4** based on the *ipso* substitution pattern in **4** and the opportunity to oxidatively functionalize the molecule. The synthesis of the oxidized pyrrolidine **6** will be approached as shown in Figure 4.

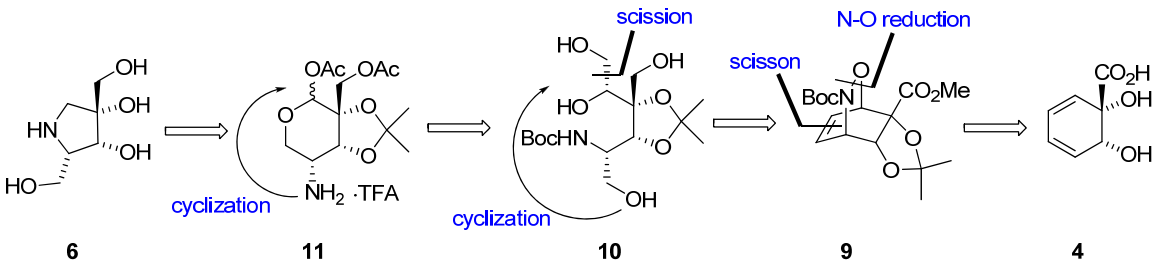


Figure 4. Retrosynthetic analysis of pyrrolidine **6**.

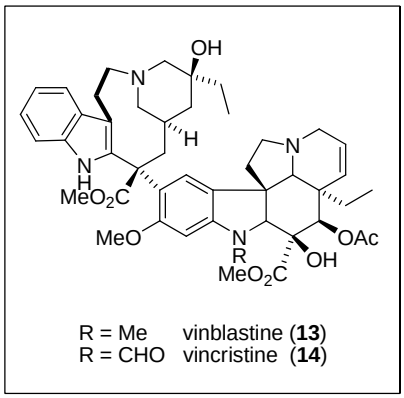


Figure 5. Vinca drugs.

The final portion of this thesis will describe the general approaches toward a Vinca alkaloid of type **12** that is contained as a structural unit in the marketed drugs vinblastine (**13**) and vincristine (**14**), Figure 5. The versatile intermediate **4**, derived enzymatically through whole-cell biocatalysis, is pre-installed with an enantioenriched quaternary center (> 95% ee) and oxidation states that match those of the natural product vindoline (**7**). It is envisaged that the tightly functionalized vindoline skeleton could be accessed by a generic tricyclic intermediate **15** by using a Fischer indolization, similar to Storks 1963 approach to *rac*-aspidospermine, Figure 6.[6] Several approaches will be outlined that include a thermally induced Diels-Alder approach of a 1-azadiene,[7] an amidyl radical approach,[8] and a tandem conjugate addition – alkylation approach.[9]

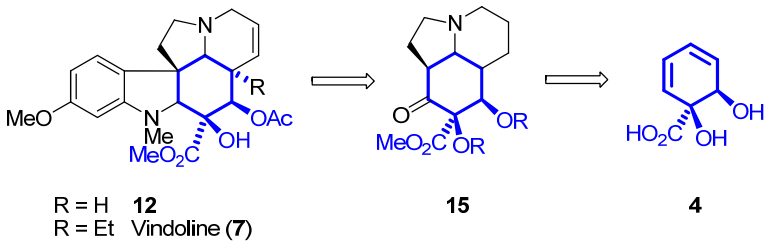


Figure 6. Retrosynethic analysis of Vindoline and Vinca derivative **12**.

2 Historical

The following historical chapter is divided into four separate sections that cover: 1. aromatic dioxygenase, 2. *Idesia Polycarpa* natural products, 3. iminosugars, and 4. Vinca and aspidosperma alkaloids. An overview of the individual fields will be presented along with selected syntheses and general synthetic strategies. A general discussion as to the biological significance of certain Vinca alkaloids, iminosugar mimics, and *Idesia Polycarpa* natural products will be addressed, as along with biosynthetic considerations.

2.1 Aromatic Dioxygenases

During the investigation into the metabolism of aromatic substrates by *Pseudomonas putida*, David Gibson isolated a *cis*-dihydrodiol metabolite from the ferment broth when *p*-chlorotoluene was fed as a substrate. Extensive research effort identified toluene dioxygenase (TDO) as the enzyme responsible for this biotransformation. The following chapter will review the more prominent organisms that express dioxygenase enzymes, namely toluene dioxygenase (TDO), naphthalene dioxygenase (NDO), and benzoate dioxygenase (BZDO). A historical prospective will precede a discussion of the substrate specificity and application of the biocatalytically derived metabolites in enantioselective synthesis.

2.1.1 History

An exact time at which the process of fermentation was founded remains uncertain. It can be discerned that at the time when mankind was developing the skills needed for sustainment, *e.g.* the domestication of livestock and the cultivation of crops,

the Neolithic man was simultaneously witnessing the natural processes of fermentation. The particular time period (ca. 5400–5000 BC) can be substantiated by excavations of a Neolithic village in the Zagros mountains of northern Iran in which a jar was unearthed that contained the calcium salt of tartaric acid and oleoresin.[10] The addition of additives, in particular the alcohol-soluble terebinth resin, alludes to the intent in preserving the contents of the vessel by inhibiting bacterial (*Acetobacter*) growth, thus preventing oxidation of ethanol to acetic acid. The realization that a natural process was occurring was evident to the early man and as civilizations developed the oxidizing nature of fermentation was harnessed to include such beneficial utilities as the production of alcoholic beverages, cheese, bread, yogurt, and other consumables to supplement dietary consumption.

Fast forward thousands of years through the evolution of culture, thought, and the development of the sciences, and you will find the founders of modern-day biology and chemistry seeking to answer fundamental questions to the transformations they observed. As early as the mid-18th century the conversion of starch to sugars by plant extracts was known and in 1752 the French scientist René Antoine Ferchault de Réaumur observed the digestion of meat by hawk stomach secretions.[11] By the 1830's the role of yeast was a heavily debated scientific topic as to whether it was a living organism equipped with self-embodied machinery or a chemical phenomenon (oxidation) made possible by the exposure of "plant juices" to air. Proponents of the former theory were scientists such as Cagniard de Latour, Schwann, and Kutzing who observed yeast cells under microscopic magnification as spherical particles capable of reproduction.[12] They regarded the alcoholic fermentation as a process of vegetative existence. Schwann even concluded that

yeast gained nourishment from the sugar while the remaining elements of the broth were not consumed as nutrient and would combine to form alcohol. He went on to denote yeast as "sugar fungus." Kützing postulated that yeast was, in fact, an organized body and not an individual compound. In 1833, French chemists Anselme Payen and Jean-Francois Persoz isolated a white precipitate from a barley malt extract. They noted that the water soluble material was able to convert starch to maltose and, thus, reported the first *in vitro* enzymatic transformation. They coined their malt extract "diastase" and established the nomenclature for naming enzymes, with the suffix *–ase*.[13]

Two decades later in 1856, Louis Pasteur was approached by a distillery and asked to help solve a problem with alcohol production *via* their sugar beetroot fermentation. Pasteur analyzed the questionable batches of ferment material and found them to contain a considerable amount of lactic acid instead of alcohol. He observed the samples under a microscope and found that the containers in which the alcoholic fermentation occurred contained a large amount of yeast cells. In contrast, the contaminated vessels that contained lactic acid contained much smaller cells than the yeast and he hypothesized that there were two separate types of fermentation involved, alcoholic and lactic acid, that occur by two distinct processes: alcoholic fermentation by the action of yeast and lactic acid fermentation by the action of bacteria.[14, 15] Not long after Pasteur identified yeast cells as the responsible entity that possessed the mysterious "ferments" did Dumas demonstrate the ability of yeast to reduce sulfur to hydrogen sulfide.[16] In 1877, the German physiologist William Kühne designated the term "enzyme" (Greek: *to leaven*) in order to differentiate the actual process from the organism (bacteria).[17] By 1886 Adrian J. Brown had reported his experimentation with

Bacterium aceti (now called *Acetobacter*) and the conversion of various chemical substances, including the transformation of ethanol to acetic acid, propanol to propanoic acid, and mannitol into fructose.[18] Brown's early accomplishments with biotransformations have been stated as a landmark with respect to the discipline of biocatalysis.[19]

In 1894 H. Emil Fischer described the "lock and key" model to depict the substrate (key)/enzyme (lock) interaction at the active site. Fischer's postulation provided a good qualitative analogy – the piecing together of geometric shapes – but failed to address the more detailed interactions responsible for promiscuous size and shape selectivity.[20] Daniel Koshland extended Fischer's model by suggesting that a dynamic relationship existed between the substrate and active site. His "induced fit" hypothesis of enzyme action was based on the ability of an enzyme to adopt different conformational changes in order to accommodate substrate and environmental interactions, such as pH and van der Waals forces.[21] The key features of the induced fit model are attractive and are generally accepted today, such as localized assistance of amino acid side chains through non-covalent bonding and the inheritent flexibility associated with large peptidic structures. The modern concept of the "induced fit" has evolved based on the premise that electrostatic effects of the active site play a large role in predefinition of a latent transition state. The ultimate result of these interactions are the stabilization of the transition state, lowering of the activation energy (E_a), and an increase in rate of reaction.[22-24]

All of the more than 4000 identified enzymes have been grouped into the Enzyme Nomenclature Classification System and are organized on the basis of the type of reaction they catalyze, Table 1.[25]

Table 1. Enzyme categories based on types of reactions they catalyze.

Enzyme Class	Selected reactions
Oxidoreductases	Reduction of C=O, C=C; reductive amination; oxidation of C-H, C=C, C-N, C-O; cofactor reduction/oxidation
Transferases	Functional group transfers such as acyl, amino, phosphoryl, methyl, glycosyl, nitro, sulfur groups
Hydrolases	Hydrolysis of esters, amides, lactones, lactams, epoxides, nitriles; esterification, amidation, lactonization, epoxidation
Lyases (synthases)	Addition of small molecules to C=C, C=N, C=O (no energy requirement from ATP)
Isomerases	Isomerizations, epimerizations, rearrangements
Ligases (synthetases)	Formation of complex chemical compounds (energy requirement from ATP cleavage)

Enzymes and the processes they catalyze have been a topic of extensive research both academically and industrially for the past half century. Modern analytical techniques are continuously improving and the technological growth of the past 50 years has allowed for great strides in the understanding of microbiology, in particular the paradigm shift from anaerobic culture (static cultures) to aerobic culture (shaking cultures and air-fed bioreactors) in the mid-twentieth century.[26] In addition, screening for microbial diversity has been adopted as a foundation to finding, or improving on, the best producers of an

enzymatic process and the introduction of new techniques, such as directed evolution and the engineering of metabolic pathways, have emerged as a result.

There is no doubt in the efficiency of biological systems to perform complex chemical transformations. It has been demonstrated that enzymatic systems are adaptable for the use in a wide range of industries that include environmental issues, food production, fine chemicals, pharmaceuticals, and many more. Some interesting and impressive examples of such robust processes will be briefly examined in section 2.1.5 of this document. Of interest to the readers of this thesis is the application of biocatalysis to the synthesis of complex molecules. Specifically, the use of *cis*-dihydrodiols isolated from the whole cell fermentation of aromatic substrates and used as raw materials for organic synthesis.

2.1.2 Arene Oxidation by Oxidoreductase Enzymes

Enzymes that are capable of dioxygen activation are divided into two subcategories: oxidases and oxygenases. Oxidases use oxygen as their oxidant and are reduced to hydrogen peroxide (H_2O_2) and water (H_2O). Oxygenases can either incorporate one of the oxygen atoms of dioxygen (mono-oxygenase) or both of the oxygen atoms of dioxygen (dioxygenase) into the substrate/product during the enzymatic transformation. Most of the known dioxygenase enzymes require a metal cofactor, usually iron(II) or iron(III), in order to establish the oxidation-reduction cycle. Eukaryotic organisms apply numerous cytochromes to perform their oxidative processes while prokaryotes utilize dioxygenase enzymes to deliver dioxygen to the substrate and affect oxidation. Of the six classes of enzymatic processes, Table 1, oxidoreductases are the

second most commonly used type of enzyme in organic synthesis, trailing hydrolases in their frequency of use toward synthetic application.

The connection between the microbial influences on aromatic compounds dates back to 1908 when Stormer reported the isolation of an organism, *Bacillus hexacarovorum*, that was capable of the assimilation of toluene and xylene.[27] Six years later Wagner reported the metabolism of toluene, xylene, benzene, and other aliphatic hydrocarbons by *Bacterium benzoli a* and *b*. *B*. *benzoli a* was also found to grow on phenol and pyrocatechol. Wagner also observed the quantitative "destruction" of crude oil samples presumed to contain naphthenic aromatic and aliphatic hydrocarbons.[28] In 1957 pyrocatechol was isolated as the major constituent of the fermentation of benzene with *Nocardia coralline* and the debate as to which intermediates were present during the oxidation followed.[29] On the basis of previous studies conducted by Ayenger *et al*.,[30] where mammalian dehydrogenation of 3,5-cyclohexadiene-1,2-diol (**16**) was observed to give pyrocatechol (**17**) and Young[31] where 1,2-dihydronaphthalene-1,2-diol (**18**) was isolated from the urine of naphthalene (**19**)-fed rats, Marr and Stone proposed that *trans*-cyclohexa-3,5-diene-1,2-diol (**20**) was a likely intermediate in the bacterial oxidation of benzene (**21**), Table 2.[32] They were able to chromatographically identify pyrocatechol obtained from culture medium of *Pseudomonas aeruginosa* that was grown on benzene as the sole carbon source. It was argued that the oxidation of benzene followed the same general metabolic pathway found in bacteria capable of the metabolism of other aromatic compounds such as phenol, benzoic acid, and naphthalene and did not include phenol or *o*-benzoquinone as probable intermediates. The proposition of an epoxide precursor in the metabolic pathway leading to various catechols[33, 34] had been stated prior to Gibson's

1968 reports on the oxidative degradation of benzene with *Pseudomonas putida*.[35] Despite reports from Holtzman *et al*., indicating that only a singley incorporated oxygen atom (^{18}O labeled) is incorporated into *trans*-1,2-dihydro-1,2-dihydroxynaphthalene (**22**) using mouse liver microsomes fed with naphthalene,[36] Gibson alluded to the *cis* addition of dioxygen to benzene as was previously suggested by Kobayashi and coworkers in the enzymatic formation of catechol from anthranilic acid (**23**), Table 2.[37] Gibson's studies dismissed the idea that catechol is generated *via* the epoxidation pathway stating that hydrolysis of epoxides invariably occur with formation of *trans*-glycols and upon feeding washed cell suspensions of *P. putida* with *trans*-3,5-cyclohexadiene-1,2-diol (**20**) insignificant rate of consumption was observed. In addition, Gibson was never able to detect the formation of phenol in the ferment broth and subsequent experiments with cell extracts that were unable to metabolize phenol as a carbon source, discrediting the notion of a monohydroxylation pathway.[35] In his follow-up communication that same year (1968), Gibson demonstrated that *P. putida* was able to oxidize halobenzene substrates to the respective 3-halogenated catechol derivatives.[38] Upon treatment of ferment broth with *p*-chlorotoluene (**24**) he was able to isolate two metabolites that were identified as (+)-*cis*-4-chloro-2,3-dihydroxy-1-methylcyclohexa-4,6-diene (**25**) and 4-chloro-2,3-dihydroxy-1-methylbenzene (**26**), Figure 7. The incubation with ^{18}O-labeled dioxygen indicated the incorporation of two atoms of atmospheric oxygen that presumably originated in the same molecule. These findings were substantiated by mass spectral analysis. It was also suggested that the dihydroxylation was iron-mediated and involved molecular oxygen.

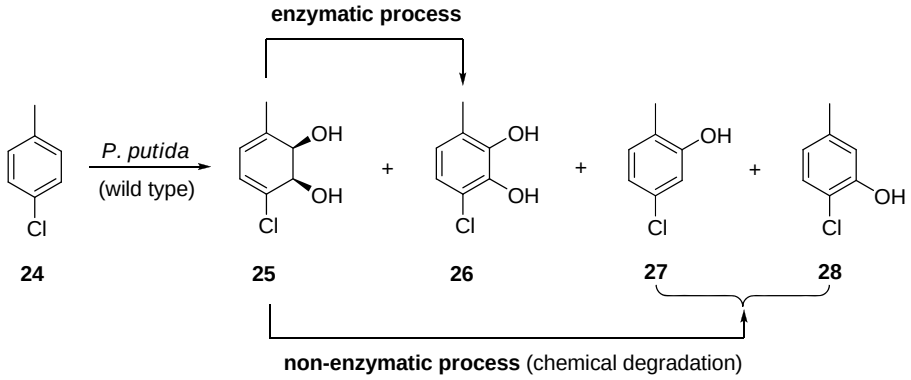


Figure 7. Metabolism of *p*-chlrotoluene **24** by *P. putida*. (Gibson)

The development of mutant strain *P. putida* 39/D by the Gibson group allowed for the accumulation of (+)-*cis*-2,3-dihydroxy-1-methylcyclohexa-4,6-diene (**29**) from the culture media when grown in the presence of toluene (**30**), Figure 8.[39]

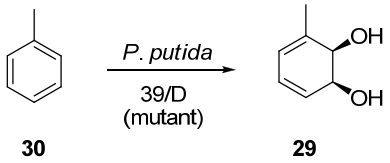


Figure 8. Metabolism of toluene by *P. putida* 39/D. (Gibson)

Analysis of metabolite **29** by nuclear magnetic resonance (NMR) did not provide unequivocal proof as to the vicinal diol stereochemistry. Gibson and coworkers prepared the cycloadduct 1-methyl-2,3-diacetoxybicyclo[2.2.2]-7-hexene-5,6-dicarboxylic anhydride (**31**) *via* acetylation and subsequent treatment with maleic anhydride, Figure 9. The hydrogenation of **31** provided the fully saturated derivative **32** and NMR analysis indicated a *cis* relationship between protons H_a and H_b ($J_{ab} = J_{ba} = 8.5$ Hz) and provided proof of relative stereochemistry.

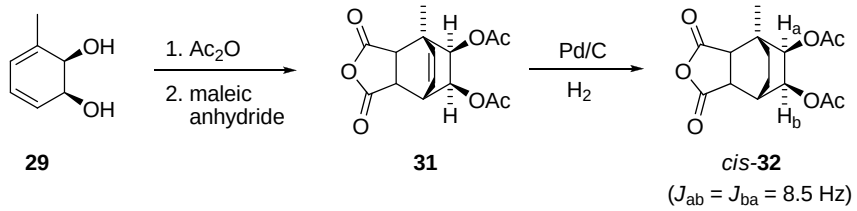


Figure 9. Relative stereochemical proof of metabolite **29**, from fermentation of toluene with *P. putida* 39/D. (Gibson)

The absolute stereochemistry of the *cis*-dihydrodiol **29** was later established[40] through a series of transformations that led to a known compound, namely (*R*)-(–)-methyladipic acid (**33**), whose absolute stereochemistry had been previously established, Figure 10.[41] The hydrogenation of **29** provided a mixture of *cis*,*trans*- and *cis*,*cis*-3-methylcyclohexane-1,2-diols, **34a** and **34b** respectively, that were separable upon derivatization as the C1 monobenzoates. The hydrolysis of the monobenzoate of **34b** and subsequent oxidative cleavage of the *vic*-diols with Jones reagent yielded (**33**). The degradation of **29** to the known **33** confirmed the stereochemistry as 1*S*,2*R*.

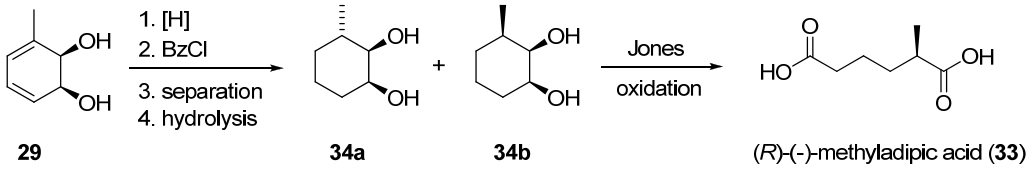


Figure 10. Absolute stereochemical proof of *P. putida* 39/D metabolite **29**. (Gibson)

The pioneering work by Gibson, which is historically relevant in the field microbiology, chemistry, and biotechnology, spurred the onset of growth toward the acceptance and applications of biocatalysis. The search for practical substrates, for both synthetically useful and environmentally applicable purposes, was initiated by many academic groups and some industrial establishments. In particular, Albey M. Reiner at the University of California at Berkeley began studies involving the mutant strain *Alcaligene eutrophus* B9 (now called *Ralstonia eutrophus* B9) that was blocked in

benzoic acid (**3**) catabolism and accumulated (–)-3,5-cyclohexadiene-1,2-diol-1-carboxylic acid [1,2-dihydro-1,2-dihydroxybenzoic acid (DHB)] (**4**) in the ferment broth, Figure 11.[42]

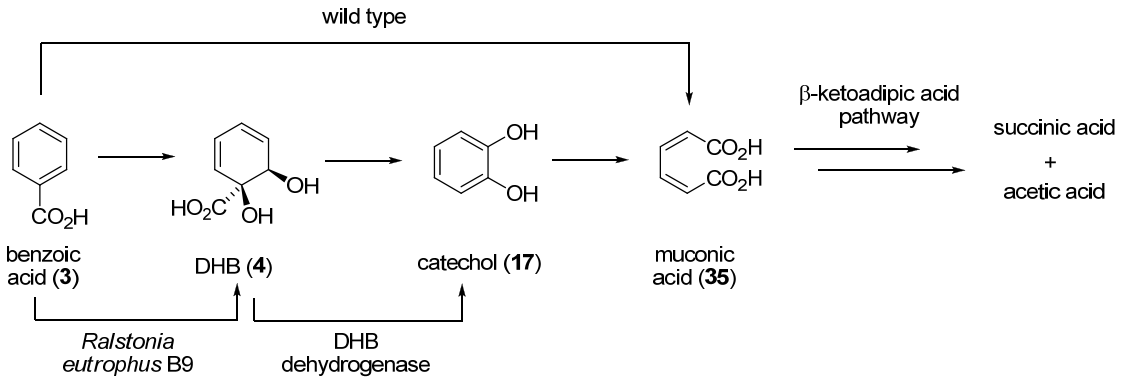


Figure 11. Metabolism of benzoic acid by mutant strains.

Reiner and coworkers confirmed the incorporation of both oxygen atoms from molecular oxygen by mass spectroscopy ^{18}O-labeling studies. He further hinted to the *cis* relationship of the vicinal hydroxyl groups by performing chemical oxidations (periodate) on the derived metabolite **4** and making rate comparisons with reference standards (*trans*- and mixed *trans*- and *cis*-cyclohexane-1,2-diol). It wasn't until 24 years after Reiner's (1971) work that the absolute stereochemistry was confirmed. Ribbons and Widdowson were implementing biocatalysis techniques, namely the mutants *R. eutrophus* B9 and *P. putida* U109,[43] for the production of useful chiral synthons. Widdowson recognized the advantage that was inherent in these enzymes for synthetic application. The absolute stereochemistry of DHB (**4**) was established by derivatization as the *p*-bromobenzoylmethyl ester **36** and analysis by X-ray diffraction indicated a 1*S*,2*R* configuration, Figure 12.[44]

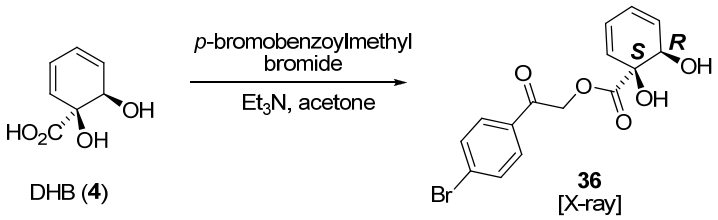
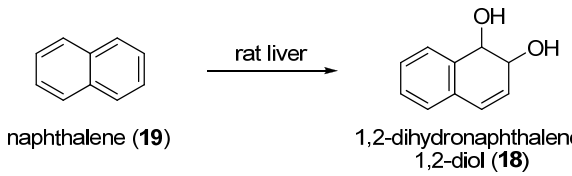
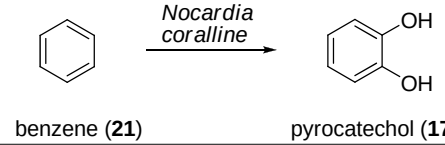
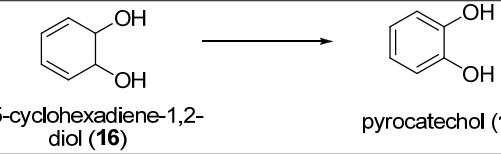
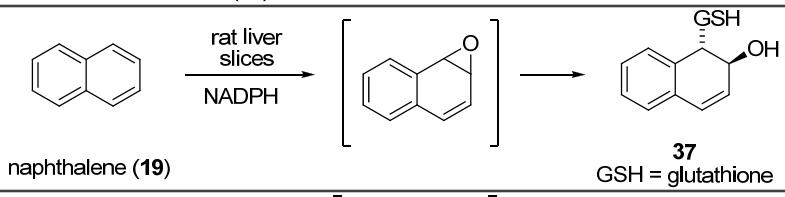
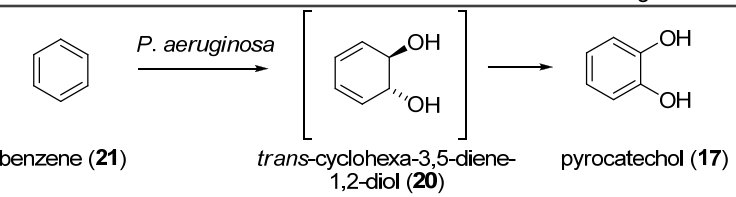
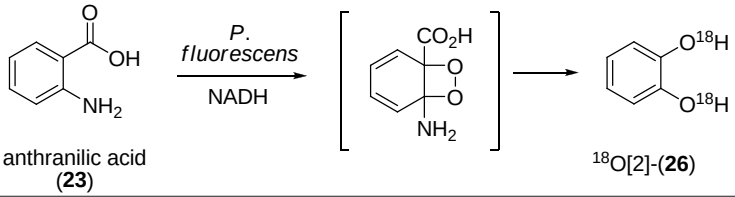
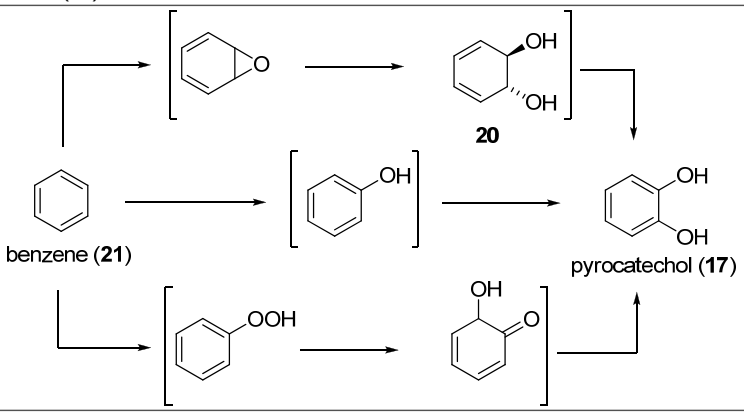
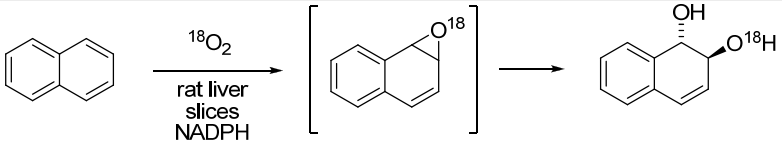
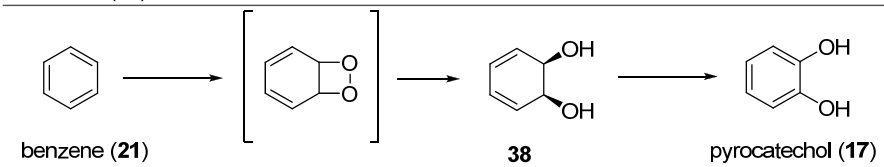
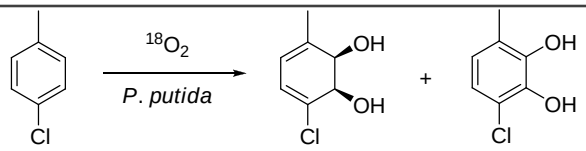
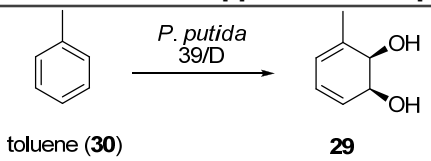
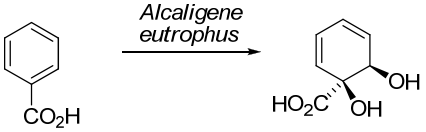


Figure 12. Absolute stereochemistry determination of DHB (**4**)

The evolution of suggested intermediates that occur during the biocatalytic oxidation of arenes have been extensively discussed. A chronological representation is presented in Table 2 and a more detailed mechanistic survey will be described in the following section.

Table 2. Chronological evolution of selected arene dioxygenase metabolites

	**Young** (1947)
	**Haccius, Helfrich** (1957)
	**Ayenger *et al.*** (1958)
	**Booth** (1960)
	**Marr, Stone** (1961)

 anthranilic acid (**23**) ^{18}O[2]-(**26**)	**Kobayashi *et al.*** (1964)
 benzene (**21**) pyrocatechol (**17**)	**O'Connor *et al.*** (1964)
 naphthalene (**19**) **22**	**Holtzman *et al.*** (1967)
 benzene (**21**) **38** pyrocatechol (**17**)	**Gibson *et al.*** (1968)
 **24** ^{18}O[2]-**25** ^{18}O[2]-**26**	**Gibson *et al.*** (1968)
 toluene (**30**) **29**	**Gibson *et al.*** (1970)
 benzoic acid (**3**) **4**	**Reiner, Hegeman** (1971)

* All bracketed intermediates were suggested and were not established by rigorous scientific methods.

2.1.3 Benzoate dioxygenase (BZDO) and Mechanistic Insight

Rieske dioxygenases (non-heme) catalyze the stereo- and regiospecific, O_2-dependent transformation of unactivated aromatic derivatives to *cis*-dihydrodiols, as discussed in Section 2.1.2. The enzymatic process provides a useful carbon source for bacterial growth. There has been much literary discussion as to how the transformation proceeds from an energetically stable aromatic molecule to a non-aromatic, chemically labile *vic*-diol. Several mechanisms have been postulated and include such processes as dioxetane **39** formation *via* singlet oxygen ($^1O_2$),[45] [3+2]-type cycloaddition involving an iron(V) peroxide species possibly going through intermediates **40** – **42**,[19] and, as a more generally accepted pathway, an iron(II)/iron(III) peroxide incorporation of molecular oxygen to yield transient iron(III) peroxo species **43** leading to *cis*-dihydrodiol **2**, Figure 13.[46-48] Some of these mechanistic approaches have been studied on model enzyme systems, in particular naphthalene dioxygenase (NDO), and have implications for many other dioxygenases.

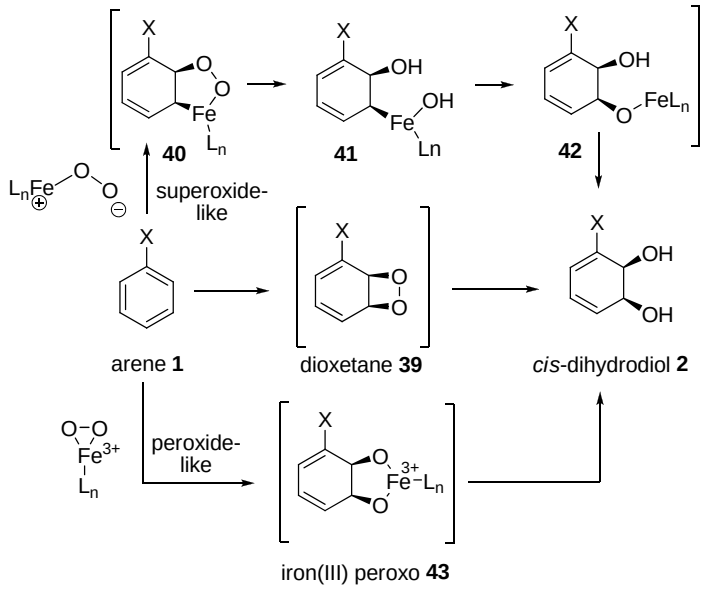


Figure 13. Mechanistic hypotheses of dioxygenases.

Benzoate dioxygenase (BZDO) is a typical Rieske dioxygenase system. The overall stoichiometry requires a single molecule of O_2 and two electrons from NADH to form the *cis*-dihydrodiol products, Figure 14. The benzoate dioxygenase system (BZDOS) consists of a reductase (BZDR) and an oxygenase (BZDO) component. BZDR is a 38 kDa protein that contains a flavin adenine dinucleotide (FAD) cofactor and a [2Fe-2S] cluster with all cysteine ligation.[49] BZDO is a 200 kDa protein of $(\alpha\beta)_3$ quaternary structure[50] and each α subunit contains a mononuclear non-heme iron active site and a Rieske-type [2Fe-2S] cluster. BZDR is reduced by NADH and transfers electrons stepwise to the Rieske cluster of the BZDO component.[47] Spectroscopic investigations indicate that oxygen activation and substrate binding occur at the mononuclear iron center of BZDO.

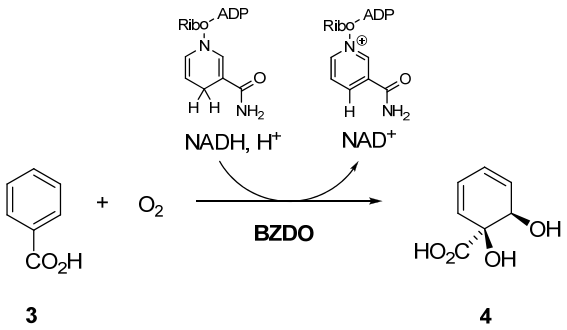


Figure 14. Benzoate dioxygenase

It is not feasible that dioxygenase enzymes could access the $^1O_2$ excited state on the basis of the 22 kcal/mol energy difference above the ground state of $^3O_2$. Three proposals were suggested for the mechanisms involving the activation of dioxygen to be legitimate: orbital overlap with a transition metal ion, single electron transfer (SET), and/or reaction with a substrate radical.[51] Singlet oxygen readily reacts with alkenes and dienes using its lone pair of valence electrons, Figure 15. The complexation of molecular oxygen to a transition metal ion containing unpaired 3d electrons could facilitate the interaction with a singlet organic reagent, in a similar way as to how sensitizers (Rose Bengal, tetraphenylporphyrin, *etc*.) are used in [4+2] cycloadditions (**44** → **45**).

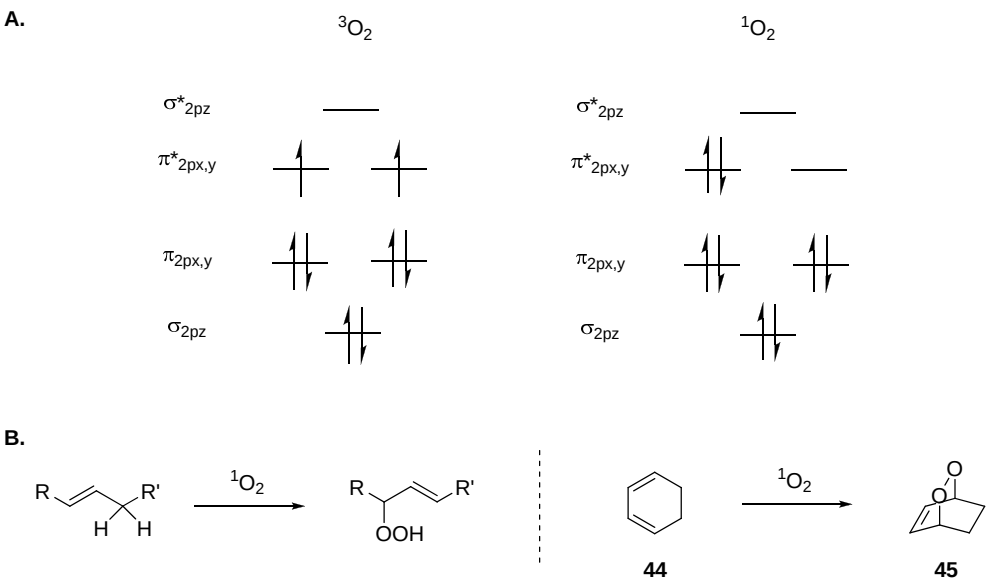


Figure 15. (A) Molecular orbital diagrams for $^3O_2$ and $^1O_2$. (B) Reactions of $^1O_2$.

For a single electron transfer to occur the metals found in dioxygen activating metallo-enzymes must have two consecutive available oxidation states (*e.g.* Fe(II)/Fe(III)) in order to affect the electron transfer to $^3O_2$ ground state and form superoxide. Uncertainty of this theory lies in the reduction potential difference between O_2/O_2^- (E_h = -0.16 V in H_2O)[52] and Fe(III)/Fe(II) (E_h = +0.77). The activation of dioxygen to superoxide by Fe(II) reduction potential is -0.93 V. Although this reduction potential is low and presents a seemingly unfavorable possibility for an event, it is likely that the active site of metallo-enzymes (redox enzymes) would influence the reduction potential of bound cofactors favorably. Examples of dioxygen activation to superoxide can be found with hemoglobin.[53] Alternatively, SET can occur with dioxygen from organic reagents that are able to form stable radical species, for instance, the formation of superoxide by reduced flavin, Figure 16.

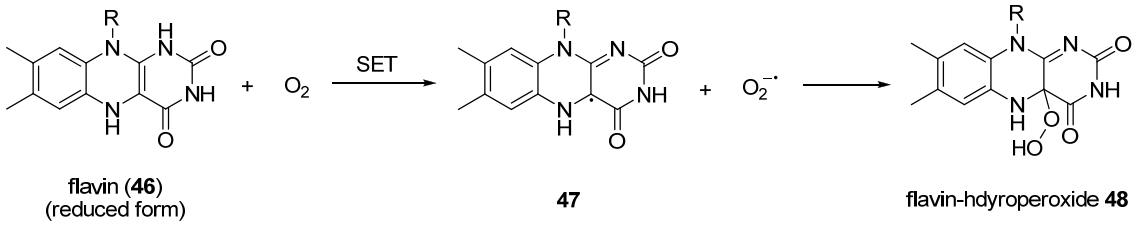


Figure 16. Dioxygen activation by reduced flavin.

Last is the possibility of a substrate radical attack onto dioxygen. This is a spin-allowed process and is proposed a mechanism for intradiol catechol dioxygenases.[54] A semiquinone intermediate reacts with dioxygen to form a hydroperoxide radical, Figure 17.

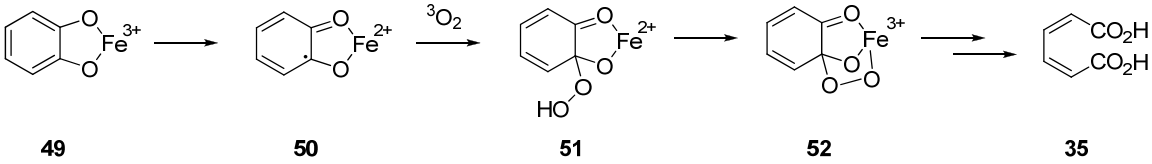


Figure 17. Substrate radical attack on dioxygen. Intradiol catechol dioxygenase example.

The mechanistic features of the BZDOS are further complicated, as in all Rieske dioxygenase systems, by the mononuclear iron center capable of multivariate ligations, *i.e.* able to coordinate to different amino acid residues. The variability of the iron coordination environment is what distinguishes the Rieske systems from the heme-based oxygenases and, thus, increases the number of possible mechanisms by which dioxygen can be activated toward oxidation of arenes.

To date, the best analogy regarding the mechanism of dioxygenases is drawn from Gibson's work on naphthalene dioxygenase (NDO). In 2003, he published the crystal structure of NDO in which the so called "side-on binding" of dioxygen to iron took place and was responsible for both of the oxygen atoms delivery to the aromatic substrate.[46] His suggested mechanism is depicted below, Figure 18, and involves a pathway

consistent with other iron(III) peroxide-like species proposed. Ultimately, the mechanism for NDO remains unsolved. However, important parallels for other dioxygenase enzymes, in particular BZDO, can be established on the basis on Gibson's work with NDO.

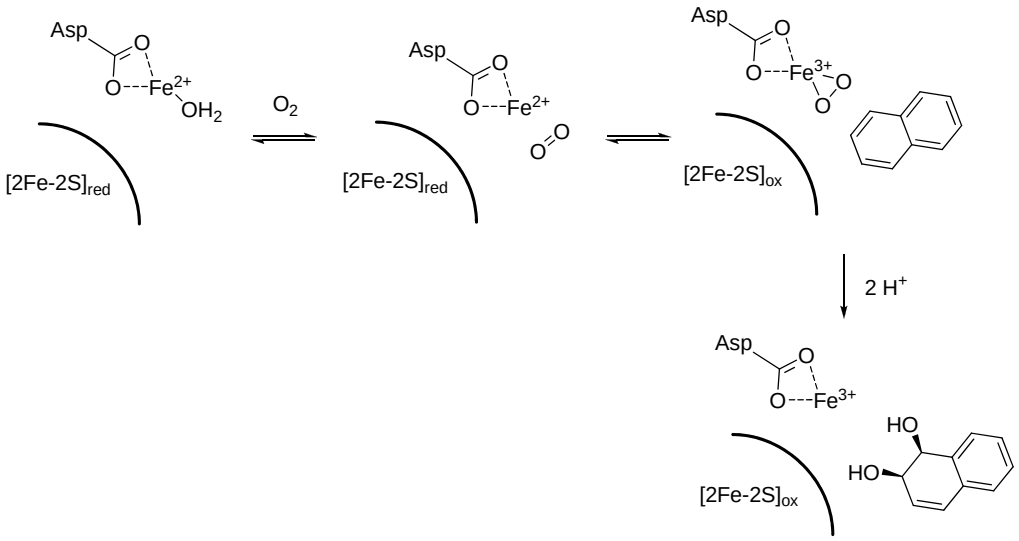


Figure 18. Gibson's mechanism based on the crystal structure of NDO.

2.1.4 Diversity of Dihydroarenediols

Boyd has derived a useful qualitative model to help predict the facial selectivity of enzymatic dihydroxylations using *P. putida* UV4, Figure 19.[55] The model depicts an environment upon which absolute stereochemistry and regiochemistry of mono- and 1,4-disubstituted benzene substrates can be estimated with good probability. The model has been successfully implemented in other dioxygenase enzymes and substituent size has proven to be a dominant factor in the selection of which bond is oxidized in the microbial system.

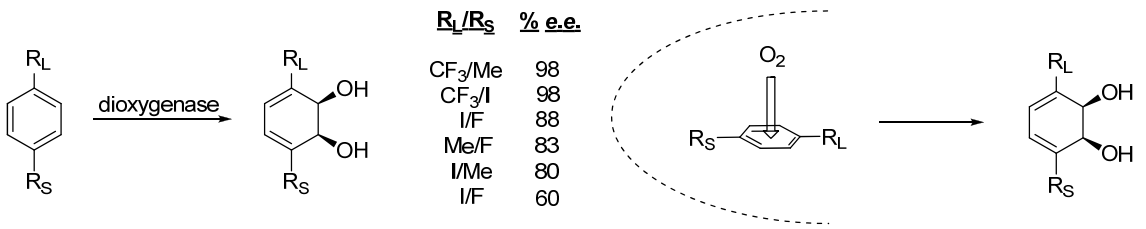


Figure 19. Boyd's model for regio- and stereochemical outcome of dioxygenase.

There are numerous examples of substrates that demonstrate scope and tolerance for selected dioxygenase enzymes.[19, 56] One of the most established systems with respect to scope is TDO, partially due to the recombinant nature of the organism, in which induction is performed from a "non-natural" inducer where metabolite contamination is avoided. Selected examples are depicted in Figure 20.

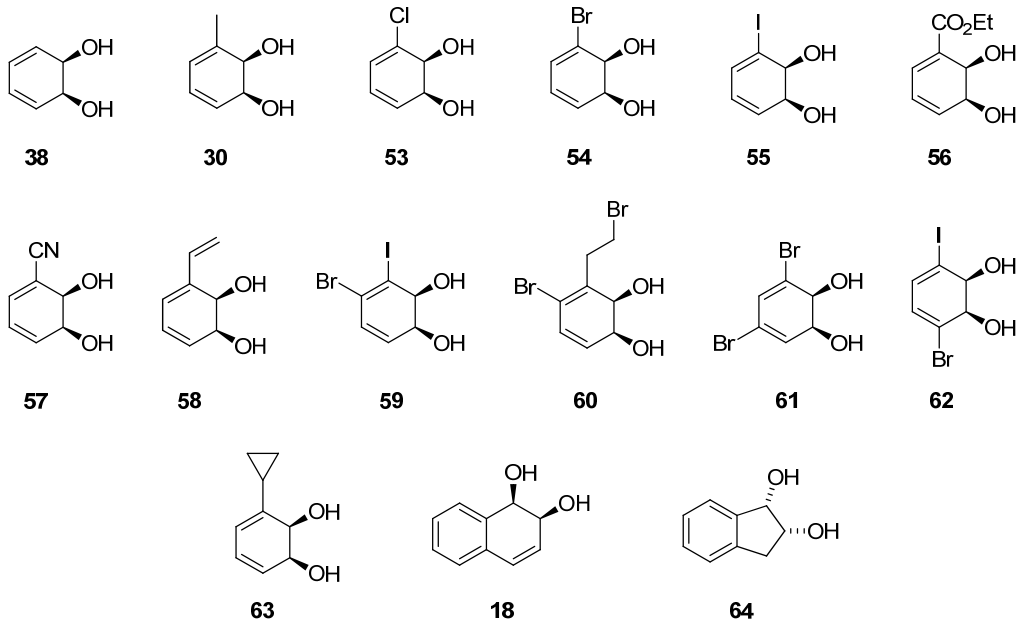


Figure 20. Selected *cis*-dihydrodiols derived from microbial oxidation (TDO, NDO).

Studies to probe the enzymatic pocket of BZDO have not been performed directly or indirectly by extensive substrate screening. The consensus that can be derived from the substrates published indicates that mono- and di-substituted benzoic acids are acceptable substrates for BZDO. *Ortho*- and *para*-substitution patterns are typically only tolerated

when fluorine is the substituent while *meta*-substitution undergoes comparatively high metabolic rates, Figure 21.

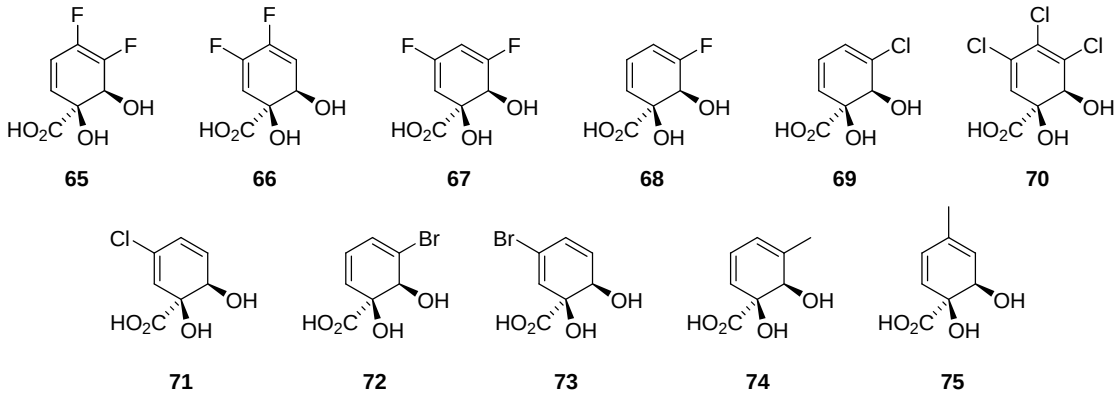


Figure 21. Selected *ipso*, *cis*-dihydrodiols derived from microbial oxidation (BZDO).

2.1.5 Utility of Dihydroarenediols in Organic Synthesis and Industrial Applications

Oxidoreductase enzymes are ideal for industrial processes where chemical oxidations/reductions methods are limited. Several factors contribute to the avoidance of redox steps in process chemistry, namely the ability to control thermal change (exotherms), toxicity or hazardous reagents that are typical of redox reagents, the financial expense to achieve selectivity through reagent control (reductions), and hazardous byproducts or waste stream generation. So why hasn't the chemical industry opted for more biocatalytic transformations? The reasons, as to the lack of acceptance in the synthetic chemistry community, have been discussed at length. However, the attitude toward biotechnology as a viable alternative in a synthetic sequence has seen much improvement in the past decade, especially in the field of agrochemicals and pharmaceutical development.

The United States Environmental Protection Agency (EPA) established the "Presidential Green Chemistry Challenge Awards" in 1995 in order to encourage and award individuals and businesses to innovate more environmentally friendly processes in their respective industries. The governing committee has established standards based on Anastas and Warner's definition of "green chemistry," Table 3.[57]

Table 3. The 12 principles of green chemistry as defined by Anastas and Warner.

1.	Prevent waste
2.	Design safer chemicals and products
3.	Design less hazardous chemical syntheses
4.	Use renewable feedstocks: Use raw materials and feedstocks
5.	Use catalysts, not stoichiometric reagents
6.	Avoid chemical derivatives
7.	Maximize atom economy
8.	Use safer solvents and reaction conditions
9.	Increase energy efficiency
10.	Minimize the potential for accidents
11.	Design chemicals and products to degrade after use
12.	Analyze in real time to prevent pollution

The 2010 winner of the Greener Reaction Conditions Award exemplifies the maturity of the field of applied biocatalysis. Merck and Codexis collaborated in the implementation of a transaminase-catalyzed formation of chiral amine **77** in their synthesis of sitagliptin phosphate (**78**), an oral type 2 diabetes drug, Figure 22.[58]

Extensive enzyme modification was performed on the transaminase by substituting 27 amino acids in order to accommodate the large substrate **79** in the active site, stabilize the enzyme to the reaction conditions, and to increase the rate of reaction. The addition of this biotransformation step eliminated four chemical steps that included a costly asymmetric hydrogenation using a rhodium catalyst in a high-pressure reactor. This example highlights the interdisciplinary cooperation between faculty in microbiology and chemistry in order to customize a chemical transformation.

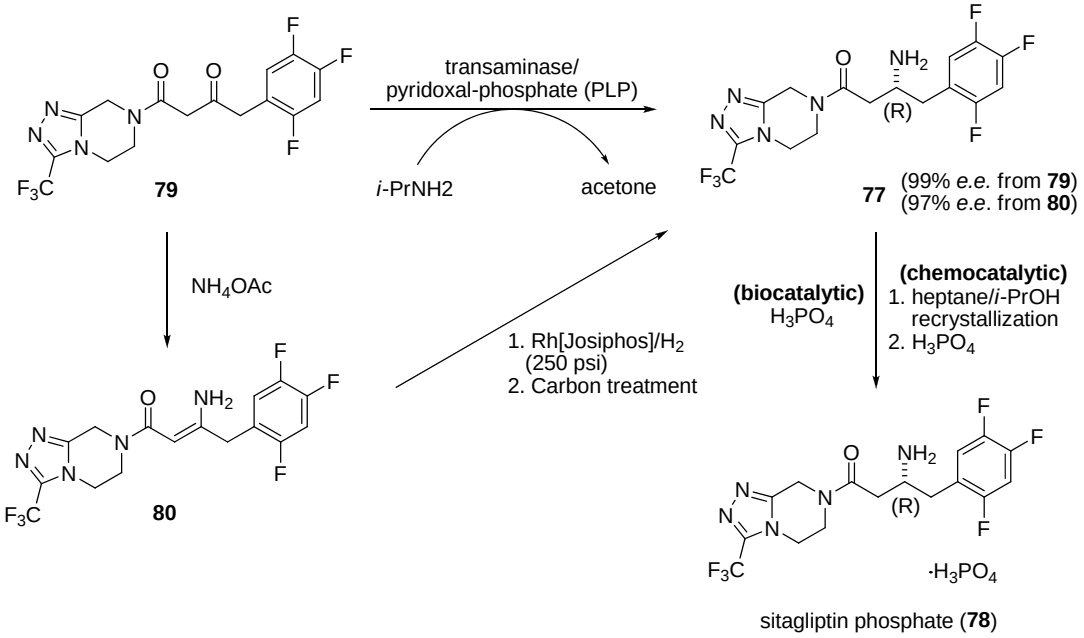


Figure 22. Merck/Codexis chemoenzymatic synthesis of sitagliptin phosphate (**78**).

The Merck – Codexis example shown in Figure 22 represents the state of the art in applied biocatalysis. The use of biotechnology in order to effect a chemical transformation has been slow to gain acceptance. It wasn't until Imperial Chemical Industries (ICI, now AkzoNobel) used *meso*, *cis*-dihydrodiol **38** derived from by microbial oxidation of benzene for the preparation of polyphenylene in 1983, Figure 23.[59]

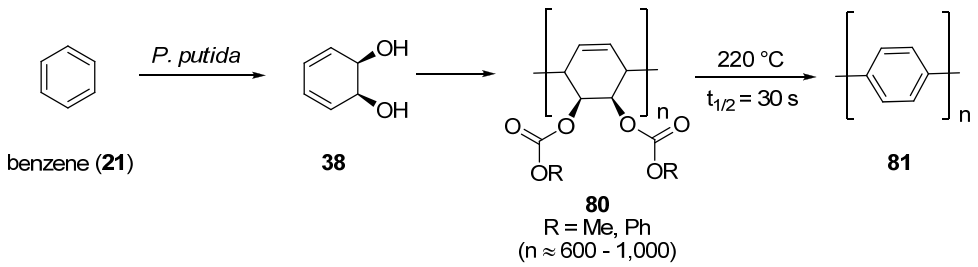


Figure 23. ICI polyphenylene synthesis using *meso*-diol **38**.

Since the ICI synthesis in 1983 several academic groups have utilized the technology to produce *cis*-dihydrodiols and implemented their versatile reactivity patterns in synthetic chemistry. Ley's group was the first academic group to take advantage of a *cis*-dihydrodiol synthon in his synthesis of (±)-pinitol (**83**).[60] His synthesis was a demonstration on how a densely oxygenated cyclohexane ring can be built through variation in synthetic construction and mode of operation. Not long after Ley's 1987 synthesis of (±)-pinitol (**83**) Hudlicky and coworkers published a concise formal synthesis of prostaglandin ($PGE_{2\alpha}$, **84**) in significantly shorter steps than any other synthesis of PGE.[61] Other, more elaborate, syntheses using microbial metabolites were to follow by a select group of academics and a representative selection is depicted below, Figure 24.[62-65]

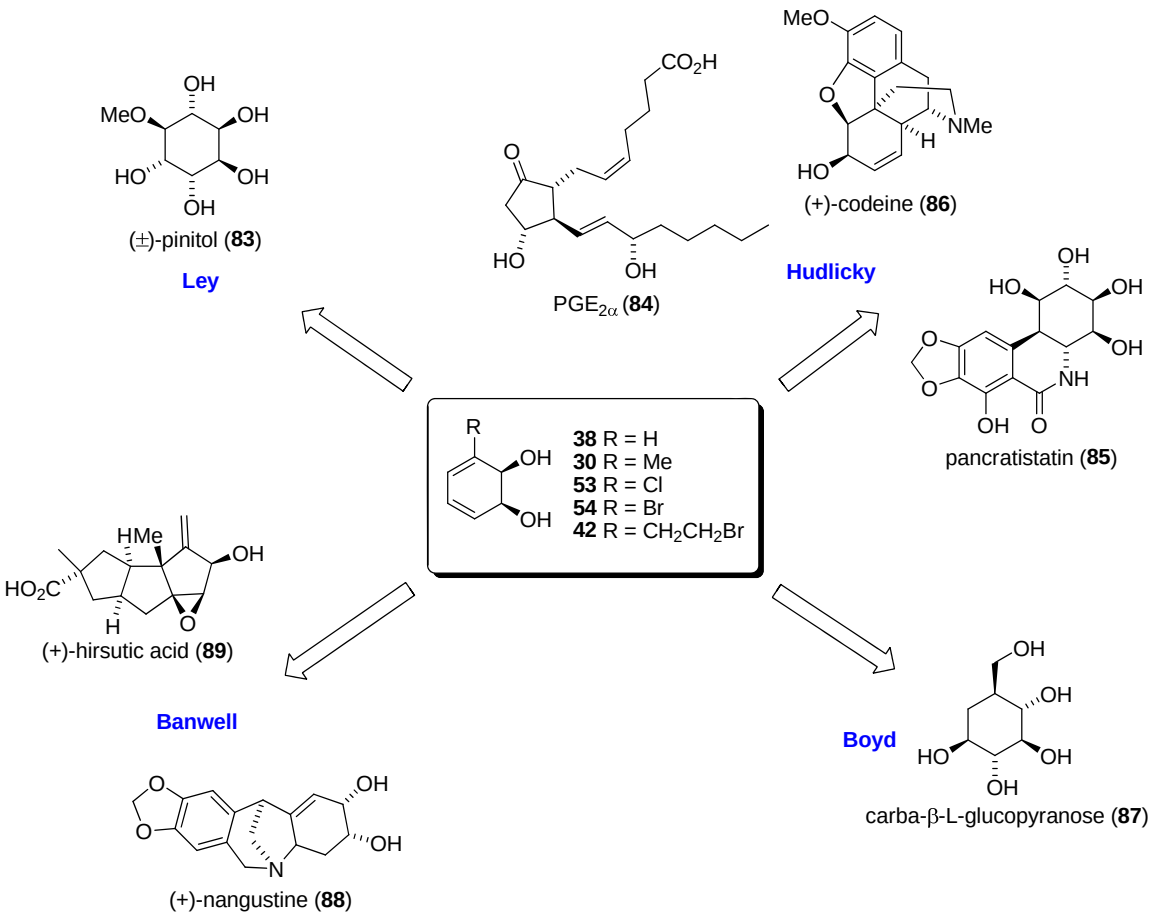


Figure 24. Diversity of synthetic targets.

The use of *ipso* diol **4** derived enzymatically from benzoic acid (**3**) has found less exposure as a synthon than the *cis*-dihydrodiols derived from halogen substituted arenes, Figure 25. However, since A. Myers' 2001 publication where he demonstrated the versatility of **2** with the generation of several useful intermediates,[66] the *ipso* substituted diol **4** has been used in several synthetic endeavors by a number of research groups, Figure 25.[67-73] The substitution pattern, namely the presence of the quaternary carbon, provides access to chemical entities otherwise difficult to obtain with good stereoselectivity.

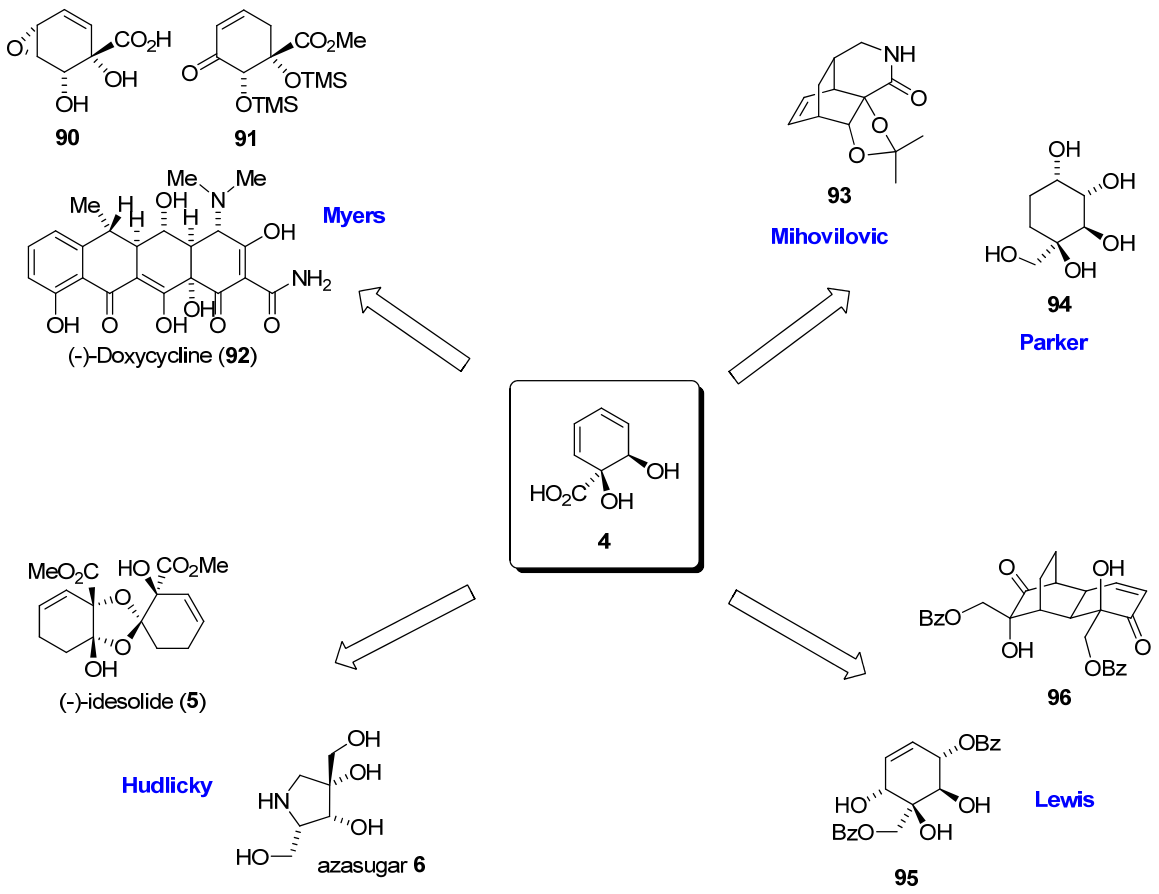


Figure 25. Diversity of synthetic targets derived from *ipso* metabolite **4**.

A more extensive survey of the application of microbial oxidation products can be found in several recent reviews and books on the topic.[19, 74-77]

2.2 *Idesia Polycarpa* Natural Products

Idesia polycarpa Maxim. is a seasonal, deciduous tree, of the Flacourtiaceae family that is native to the Asian countries of Korea, China, Japan, and Taiwan.[78] The tree is typically found at medium altitudes and a variety of compounds are present in its fruits, including fatty acids, phenazine (**97**) and derivatives,[79] pyrocatechol (**17**) , benzyl alcohol (**98**), phenethyl alcohol (**99**), idesin (**100**) and other glucopyranosides, idesolide (**5**) and its monomer **8**,[5] and flavonoids, Figure 26.[80] It bears red-colored fruit in the winter that are noticeably left untouched by wildlife. This observation is what has prompted the investigation into what compounds are present in its fruit.

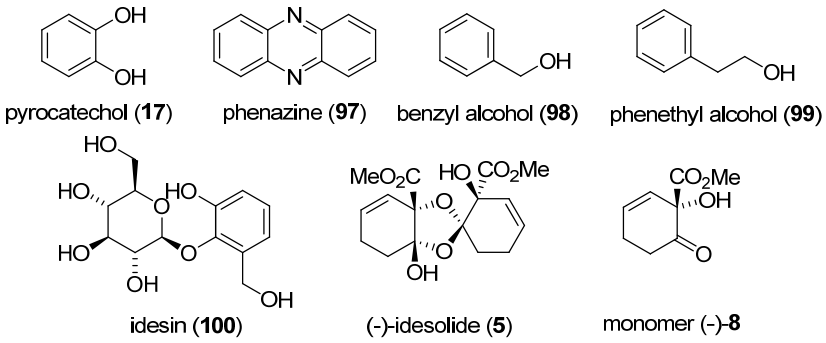


Figure 26. Compounds extracted from the fruits of *I. polycarpa*.

In 2005, Kim and coworkers from Seoul National University elucidated the structure of (-)-idesolide (**5**) by X-ray crystallography and established the relative stereochemistry, Figure 27.[5] Following the isolation of (-)-idesolide (**5**) one report on the synthesis of the monomeric unit **8**[81]of idesolide was reported and two total syntheses[82, 83] were published. All three reports, in addition to the synthesis by the Hudlicky group, will be discussed in the following sections.

Figure 27. ORTEP rendering of X-ray crystallographic data for (-)-idesolide (**5**).

2.2.1 Isolation and Characterization

Although no reports indicate that idesolide **5** is found in any other natural source other than *I. polycarpa*, its monomeric unit **8** has been isolated from the leaves of *Dovyalis abyssinica* native to Central Africa and northern South America[84] and from the twigs and stems of *Homalium ceylanicum* found in Thailand.[85] The monomeric unit has been known since 1970 when Darling *et al*. isolated and characterized glucopyranosides salicortin (**101**) and the benzoate derivative tremulacin (**102**) from the bark and leaves of *Salix purpurea*, Figure 28.[86, 87] The monomer **8** is present as a subunit of the glucosides but was never isolated in its pure form in the studies performed by Darling. It was suggested as transient intermediate when salicortin (**101**) was subjected to base-catalyzed hydrolysis to produce pyrocatechol (**17**) *via* decarboxylation and aromatization.

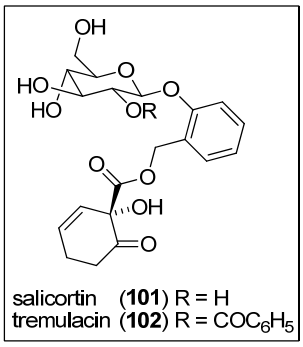


Figure 28. Monomer **8** derivatives.

In 1993 the cyclohexenyl monomer **8** was isolated in pure form from the alcoholic (*n*-BuOH) extracts of *Homalium ceylanicum* and a structure was assigned based on high

resolution mass spectrometry (HRMS *m*/*z* 171.0576, $C_8H_7O_3$) and NMR data. The effort to establish the absolute stereochemistry was hampered by the amount of material available for their investigation.[85] The *S*-configuration of **8** was not elucidated until 2010 when Iwabuchi and coworkers published their report on (-)-idesolide (**5**) by means of enantioselective synthesis.[82]

Idesolide was obtained from the methanol (MeOH) extracts of the fruits of *I. polycarpa*. Several iterations of chromatographic purifications were required to obtain an analytically pure sample of the natural product. Idesolide was fully characterized by NMR, mass spectrometry (MS), infrared spectroscopy (IR), and X-ray crystallography, Figure 27. The following physical characteristics were attributed to idesolide (**5**): melting point (mp) = 141 – 143 ˚C, $[\alpha]^{25}_D$ = -230.0 (*c* 1.0, $CHCl_3$).[5] As with the monomer **8**, idesolide (**5**) was not assigned an absolute configuration until Iwabuchi's enantioselective synthesis was reported.

2.2.2 Biological Activity Profile

Upon screening of natural products capable of exhibiting anti-inflammatory properties, Kim and coworkers identified the methanol extracts of *I. polycarpa* to effectively inhibit nitric oxide (NO) production induced by lipopolysaccharide (LPS) in BV2 microglial cells.[5] Lipopolysaccharides are the prototypical example of an endotoxin and consist of a polysaccharide and lipid portion joined together covalently. They form the outer membrane of Gram-negative bacteria and are a crucial aspect in terms of the structural integrity of the bacteria. Endotoxins elicit a strong immune response by binding to receptors on immune cells upon which cause a signaling chain in the form of cytokines, in particular pro-inflammatory cytokines in B cells, and nitric oxide

production. B cells are critical to the immune system because of their role in making antibodies in response to antigens and subsequent development into "memory B cells." After activation by an antigen, the memory B cell is capable of rapidly recognizing the same pathogen and can quickly produce the antibody in order to defend against future infection. Compromising the ability of the immune system to properly regulate antibody production is a dangerous situation, particularly in microglial cells (inflammatory cells) which act as the front line defenders of the central nervous system (CNS). Idesolide (10 μM) was reportedly able to decrease nitric oxide production in BV2 microglial cells that were exposed to LPS (100 ng/mL) for 24 hours from 19.1 μM concentration (LPS reference standard) to 4.9 μM, Table 4, thus, showing suppressed microglial cell activity indicating an improved role on the brain immune system.

Table 4. Effect of idesolide on LPS-induced NO production in BV2 microglial cells.[5]

Substrate	Concentration (μM)	Nitrite (μM)
control	-	1.7 ± 0.2
LPS	-	19.1 ± 1.3
idesolide	0.1	17.6 ± 0.7
idesolide	1.0	16.4 ± 1.5
idesolide	10.0	4.9 ± 0.1

Prolonged or excessive activation of microglial cells, through brain injury, for example, is reported to play a pivotal role in the initiation and progression of neurodegenerative diseases such as Alzheimer's and Parkinson's.[88] Effort has been devoted to improve human cognitive function while minimizing side effects. Some of this research has utilized herbal medicines such as *Ginko biboba* and Ginseng and the effects

of these extracts on neurons, synaptic functions, and the brain have shown some promise.[89] Kaang and coworkers hypothesized that idesolide, having shown inhibitory nitric oxide (NO) production in microglial cells, might be capable of improving hippocampus-dependent memory recognition in rodents.[90] Researchers used the "novel object recognition task" method[91] to gauge cognitive memory enhancements after injecting mice with idesolide (40 μg/kg). During the "sample phase" of this method the mice are exposed to two identical inanimate objects for 15 minutes (post injection). Twenty four hours after the sample phase the mice were subjected to the "retention phase" in which two objects were brought to the mice for 10 minutes, one of the objects was identical to the sample phase. The amount of time the mice spent exploring the two objects were recorded separately and, typically, preference to the novel object is observed and is perceived as an indicator of memory extent. In this study the outcome revealed that the mice treated with idesolide had a significantly higher level of recognition memory in the retention phase manifested as an increased exploration time of the novel object (49.1 min ± 6.1% for idesolide treated mice *vs*. 19.8 min ± 2.8%). The mechanisms of the effect of idesolide was not elucidated but the study suggests that idesolide may have some memory-enhancing effects without adverse side effects. The anti-inflammatory action of idesolide on the neural immune system might be the underlying facet to its therapeutic potency.

Other studies that have probed the biological activity of idesolide include its ability to increase resistance to hydrogen peroxide (H_2O_2)-induced oxidative stress in muscle cells.[92]

A 2012 study investigated the ability of idesolide to inhibit nitric oxide production and its effect on adipocyte differentiation.[93] Obesity has been linked to many additional health problems, including diabetes, hypertension, and atherosclerosis. Adipose tissue (body fat) serves as a site of calorie storage. Aside from adipose tissue's central role as lipid storage it also serves as a component of the endocrine system by secreting adipokines, such as leptin and adiponectin, that are involved in the lipid metabolism process, insulin sensitivity, and energy regulation.[94] Adipocyte differentiation is regulated by sequential activation of many transcription factors, including peroxisome proliferator-activated receptor γ (PPARγ). Inducible nitric oxide synthase (iNOS) and endothelial nitric oxide synthase (eNOS) are involved in the NO production in adipose tissue and expression has been shown to increase for adipose, muscle, and liver tissues in obese animal models.[95] The idea was to determine if blockage of adipocyte differentiation would be beneficial for the treatment of metabolic disorders. The results of the study, in fact, showed that idesolide significantly suppressed adipogenic differentiation by inhibition of NO production *via* the suppression of iNOS gene expression and the authors suggest idesolide as a potential compound for anti-obesity therapy.

2.2.3 Syntheses

Snider (2007)

Snider reported the first synthetic approach to idesolide on the premise that α-hydroxyketones undergo dimerization under mild conditions, Figure 29.[81] The spiro-bis-pinnaketal **103** was elucidated by X-ray crystallography and was determined to be an artifact of silica-gel promoted dimerization of laurencione (**104**) and not a natural product, Figure 29.[96] However, the structure of **103** was analogous to idesolide (**5**) and prompted a synthetic strategy targeting idesolide's monomeric unit **8**.

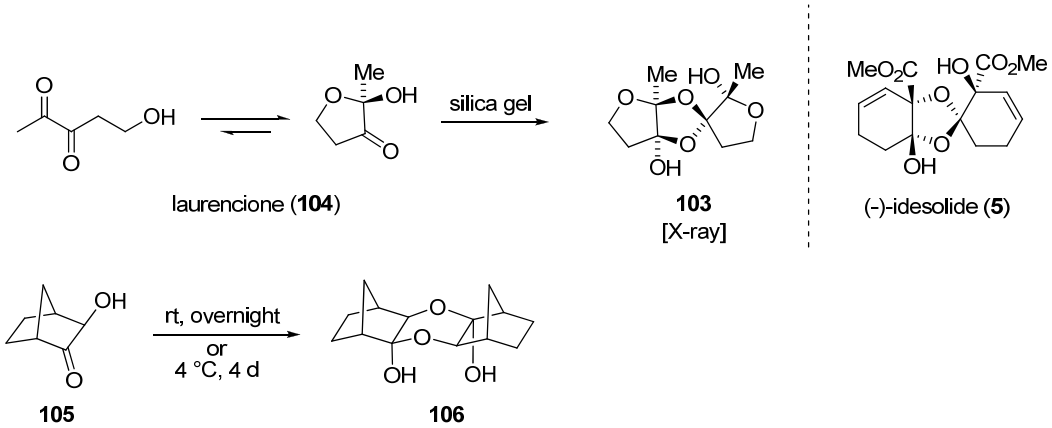


Figure 29. Dimerization of α-hydroxyketones.

Snider's synthesis started with the Birch reduction/oxidation protocol using camphorsulfonyl oxaziridine (CSO) as the hydroxylation source, a method developed by Schultz to perform asymmetric oxidations.[97] The authors noted the unstable nature of **8** and the tendency of non-conjugated diene of type **107/110/113** to aromatize, the enol ether protecting group selection became a pivotal decision on the basis of the projected deprotection conditions, Figure 30.

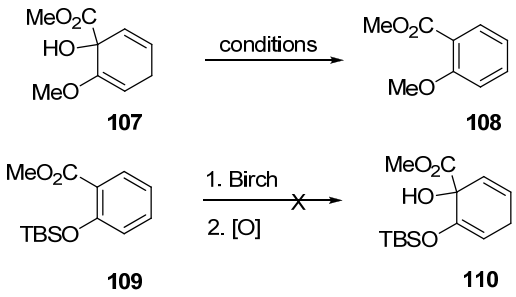
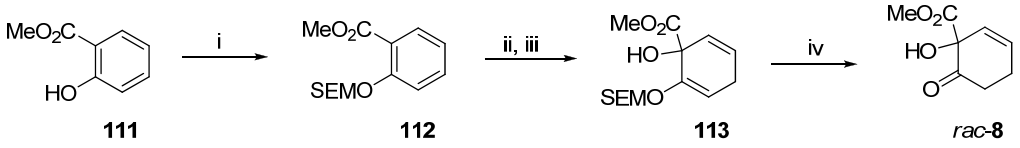


Figure 30. Protecting group selection for Birch reduction/oxidation.

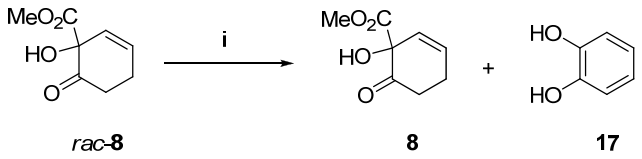
The 2-(trimethylsilyl)ethoxy methyl (SEM) ether **112** underwent the Birch reduction/hydroxylation sequence in moderate yield but with lower enantiomeric excess (nearly racemic, $[\alpha]_D = -7$; natural **8** $[\alpha]_D = -185$) than was observed with methyl ether **107** (30% *e.e.*). Monomer **8** was confirmed by X-ray analysis.



Reagents: (i) SEMCl, DIPEA, CH_2Cl_2, rt, 20h (99%); (ii) Li/NH_3; (iii) (-)-CSO (55%, 2 steps); (iv) $MgBr_2{\cdot}OEt_2$, Et_2O, $MeNO_2$, rt (71%).

Scheme 1. Snider's synthesis of α-hydroxyketone **8**.

Unfortunately, Snider and coworkers were unable to observe the expected dimerization of **8** under a variety of conditions, both as the racemate and with an optically enriched **8** (85% *e.e.*): neat (0 – 100 ˚C), D_2O, D_2O and magnesium chloride ($MgCl_2$). Optically pure **8** was obtained in 10% yield through hydrolytic kinetic resolution using pig liver esterase (PLE), Scheme 2.[98]



Reagent: (i) PLE, H_2O, 6 °C, pH = 7, 5 - 10 d (10%, 85% e.e.)

Scheme 2. Kinetic resolution of **8**.

Iwabuchi (2010)

The Iwabuchi group at Tohoku University investigated the synthesis of idesolide and had encountered the same difficulty that Snider *et al*. observed with the dimerization of **8**.[82] Iwabuchi's synthesis utilized an enantioselective oxidative kinetic resolution (OKR) with a chirally modified 2-azaadamantane *N*-oxyl (AZADO) based oxidant developed in his laboratory.[99] The chiral AZADO derivatives have shown good selectivity for the oxidation of secondary alcohols using trichloroisocyanuric acid (TCCA) as secondary oxidant, Figure 31.

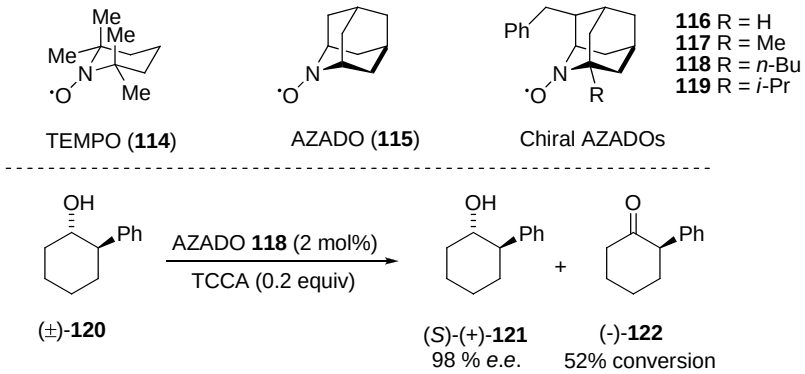
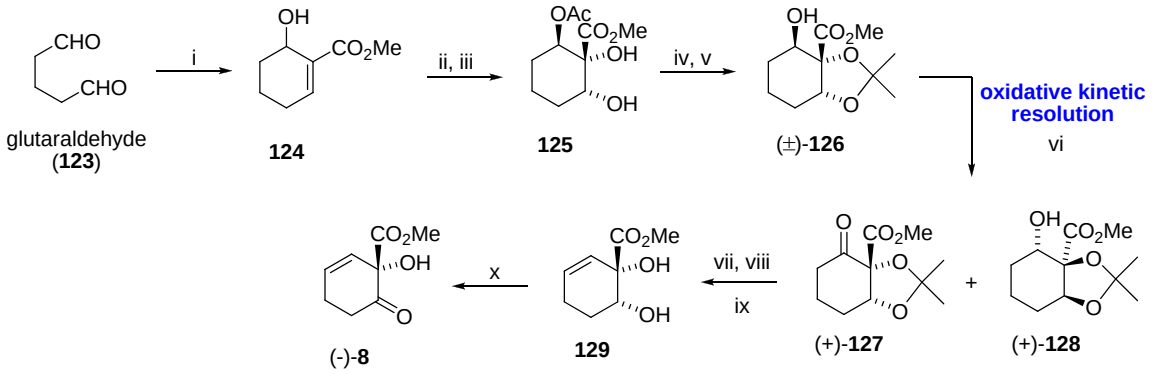


Figure 31. Iwabuchi AZADO oxidative kinetic resolution.

The synthesis demonstrates the use of chiral organocatalysis (AZADO-H) derivative **118** toward OKR and provides enantiomerically enriched key intermediate (+)-**127**. Commin's reagent was used to generate the enol triflate of (+)-**127** (not shown) and palladium catalyzed reduction with formic acid and subsequent hydrolysis of the

dioxolane provided *cis*-diol **129**. Oxidation of **129** was stated as being troublesome but was achieved with AZADO (**115**) with polymer-supported bis(acetoxy)iodobenzene (PS-BAIB)[100] as the bulk oxidant to afford monomer **(-)-8**. Apparently the use of non-polymer-supported bis(acetoxy)iodobenzene (BAIB) caused oxidative cleave of the vicinal diols in **129**.

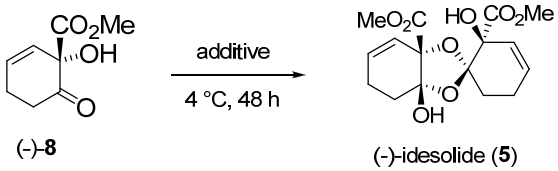


Reagents: (i) $(MeO)_2P(O)CH_2CO_2Me$, K_2CO_3 (aq); (ii) Ac_2O, DMAP, pyr, CH_2Cl_2 (45%, 2 steps); (iii) OsO_4 (cat.), NMO, THF/H_2O (95%); (iv) 2,2-dimethoxypropane, PPTS, DMF (94%); (v) K_2CO_3, MeOH (91%); (vi) AZADO **118** (5 mol%), TCCA (0.25 equiv), $NaHCO_3$ (2.0 equiv), CH_2Cl_2, -40 °C (37% **127**, 95% *e.e.*; 59% **128**, 63% *e.e.*); (vii) $PhNTf_2$, KHMDS, THF, -40 °C to 0 °C; (viii) HCO_2H, $Pd(OAc)_2$, PPh_3, NBu_3, DMF, 60 °C (63%, 2 steps from (+)-**127**); (ix) *p*-TsOH, THF/H_2O/HOAc (85%); (x) AZADO **115**, PS-BAIB, CH_2Cl_2 (71%).

Scheme 3. Iwabuchi's synthesis of monomer (-)-**8**.

Iwabuchi and coworkers confirmed the lack of tendency of monomer **8** to dimerize under neutral conditions. They subjected the monomer to ultrahigh pressure (0.8 GPa) with no success. The treatment of **8** under acidic conditions (*p*-TsOH, $BF_3{\cdot}OEt_3$) only induced dehydration to methyl salicylate **130** and basic conditions (NaH, NaOMe, DBU) led to decomposition. The serendipitous discovery of crystalline idesolide in a flask that contained monomer **(-)-8** and was contaminated with AZADO (**115**) led to the investigation of the *N*-oxyl radical species as a promoter for the dimerization, Table 5.

Table 5. Dimerization of monomer (-)-**8**.[82]



(-)-**8** (-)-idesolide (**5**)

Entry	Additive (equiv)	Solvent ($CDCl_3$)	Idesolide (%)
1	none	none	0
2	AZADO (1.0)	0.1 M	6
3	AZADO (1.0)	0.4 M	35
4	AZADO (1.0)	2 M	62
5	AZADO (1.0)	10 M	68
6	AZADO (1.0)	none	73
7	AZADO (0.1)	none	38
8	AZADO-H (1.0)	none	38
9	1-Me- AZADO (1.0)	none	63
10	TEMPO (1.0)	none	0
11	DMAP (1.0)	none	65
12	pyridine (1.0)	none	0
13	Et_3N (1.0)	none	65
14	*i*-Pr_2NEt (1.0)	none	50
15	$DMAPO{\cdot}H_2O$	none	22
16	NMO (1.0)	none	11

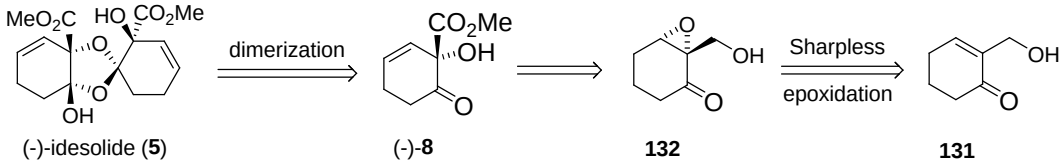
* All yields are based on ^1H NMR calculation.

As Table 5 indicates, a good conversion of monomer to its dimer is observed with stoichiometric amounts of AZADO (**115**) (entries 5 and 6). It also appeared as if AZADO was exhibiting catalytic activity (entry 7) while the reduced form AZADO-H displayed

diminished productivity (entry 8). TEMPO appeared to be too bulky about the *N*-oxyl group to be an effective oxidant (entry 10). Triethylamine (Et_3N), diisopropyl ethylamine (*i*-Pr_2NEt), and dimethylamino pyridine (DMAP) all showed the preponderance to induce dimerization while pyridine (py) did not, implicating hybridization (sp^3 *vs*. sp^2) as an important factor in the process. Iwabuchi attributed the effectiveness of AZADO in the dimerization of monomer **8** to idesolide (**5**) (34% isolated yield) to the less-hindered and basic nature of the *N*-oxyl moiety. The absolute stereochemistry of idesolide (-)-**5** was also established during the course of this investigation as 2*R*,2'*S*,3a*S*,7a*R,* Figure 21.

Kuwahara (2010)

Kuwahara and coworkers[83] followed Iwabuchi's synthesis shortly after in 2010 with a strategy based on the introduction of chirality *via* the Sharpless asymmetric epoxidation, Figure 32.[101] The starting material **131** was prepared by Baylis-Hillman reaction of 2-cyclohexenone and formaldehyde.[102]



(-)-idesolide (**5**) (-)-**8** **132** **131**

Figure 32. Kuwahara retrosynthetic analysis of (-)-idesolide.

The initial strategy was to quickly assemble monomer **8** through a ring opening elimination reaction of **132**, prepared by a Sharpless asymmetric epoxidation of **131** (92% e.e.), oxidation of the primary alcohol to the carboxylic acid, and the subsequent esterification with diazomethane to yield **133**, Scheme 4. Unfortunately, the expeditious route was unsuccessful and complex mixtures were generated when **133** was subjected to a variety of basic and Lewis acidic conditions. Trace amounts of the desired **8** were

observed in some of the reaction mixtures that contained methyl salicylate **130**, an indication that ketone enolization and subsequent aromatization was occuring, *i.e.* **136** → **130**, Figure 33.

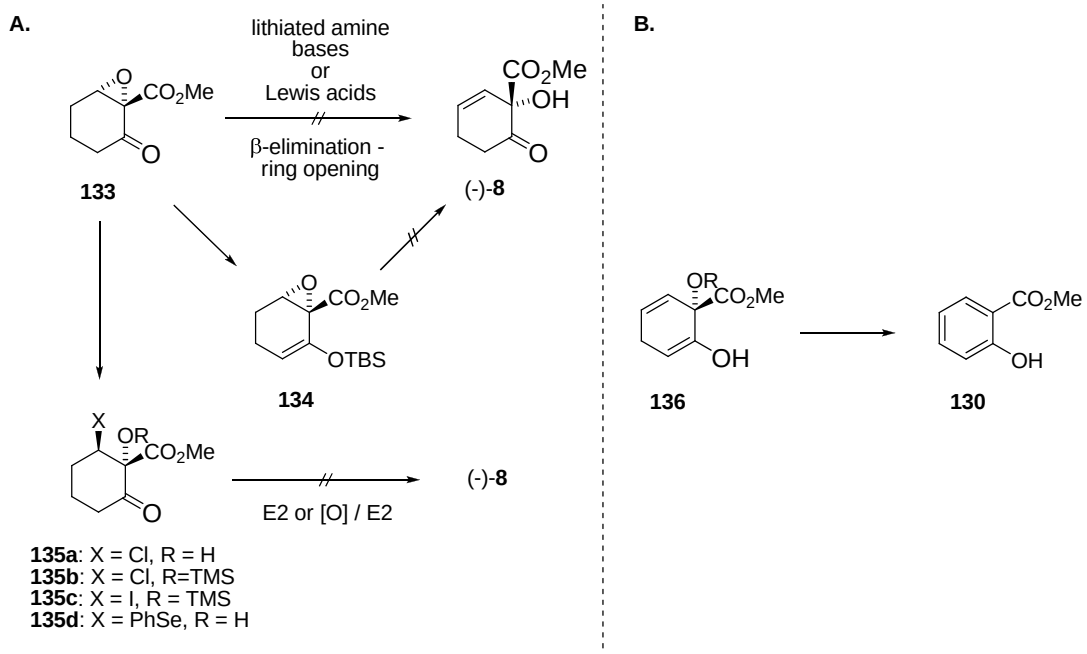
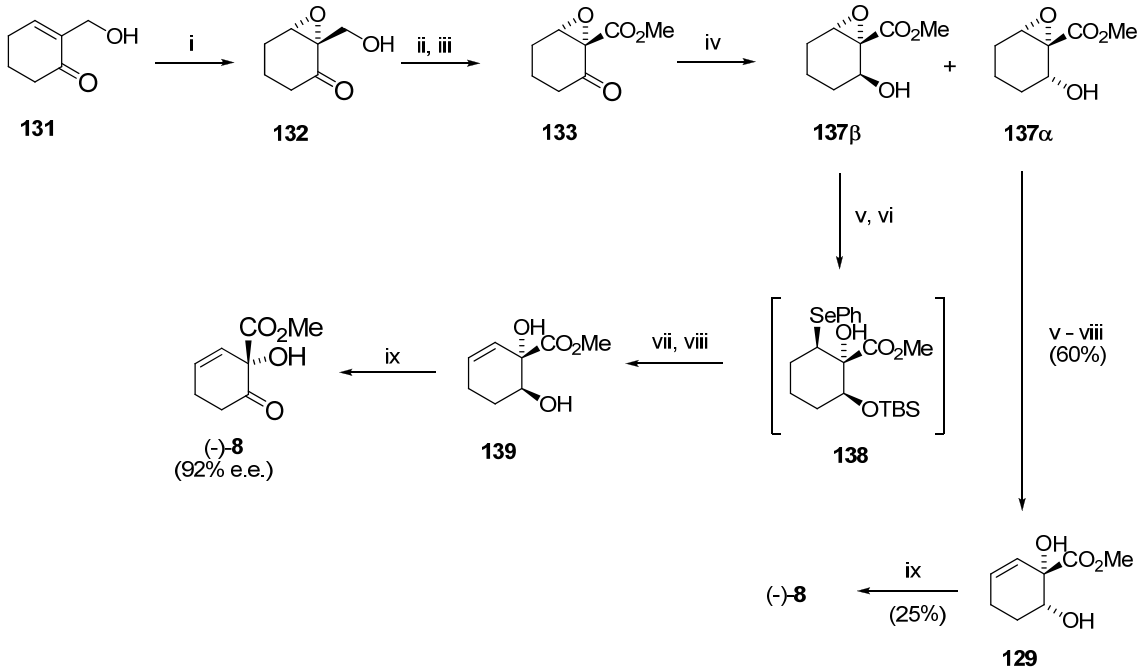


Figure 33. (A) Attempts at the conversion of **133** to (-)-**8**. (B) Suggested intermediate for decomposition of **136** to **130**.

Alternatively, the ketone in **133** was reduced in order to circumvent the problem of enolization that was suspected to be the driving force behind rearomatization. The reduction of **133** was performed in good stereoselectivity using L-Selectride (LiB(*s*-Bu)$_3$H) providing stereoisomers **137β** and **137α** in a 5.2:1 ratio that were separable by chromatographic techniques, Scheme 4. The distal hydroxyl group in **137β** was protected as the silyl ether, the epoxide was opened upon treatment with *in situ* generated phenyl selenide anion to generate **138**.[103] A one-pot oxidative elimination of selenide **138,** desilylation, and subsequent oxidation of the secondary alcohol of **139** yielded the enantioenriched monomer **8**. Diastereomer **137α** could be subjected to the same reaction

sequence as **137β** to provide **8** in somewhat lower overall yield, partially because of the tendency of the vicinal *cis*-diol **129** to oxidatively cleave upon oxidation.

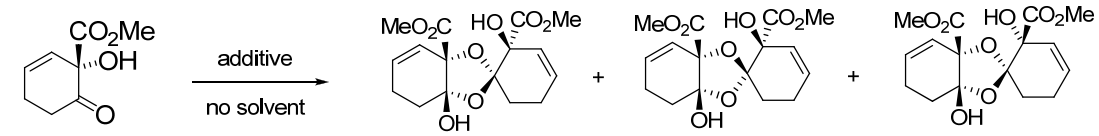


Reagents: (**i**) L-(+)-DIPT, $Ti(O\text{-}iPr)_4$, TBHP, MS 4Å, CH_2Cl_2 (92% e.e., 87%); (**ii**) CrO_3, H_2SO_4, acetone; (**iii**) CH_2N_2, ether (71%, 2 steps); (**iv**) $LiB(s\text{-}Bu)_3H$, THF (83% **137β**, 16% **137α**); Sequence for **137β** to **(-)-8**: (**v**) TBSOTf, Et_3N, CH_2Cl_2 (96%); (**vi**) PhSeSePh, $NaBH_4$, EtOH; (**vii**) $NaIO_4$, $NaHCO_3$ (92% e.e., 89%, 2 steps); (**viii**) HF (aq), CH_3CN; (**ix**) DMP, $NaHCO_3$, CH_2Cl_2 (76%, 2 steps).

Scheme 4. Synthesis of monomer (-)-**8**.

In contrast to Iwabuchi's dimerization studies, in which amine bases were primarily used, Kuwahara focused on the use of inorganic salts as a dimerization promoter, Table 6. Their investigation used inorganic additives under neat reaction conditions suggested that the site of reaction is ascribable to an increase in the interfacial area between the substrate oil/liquid and solid additive, as there was a correlation with increased yield as the amount of sodium bicarbonate was increased.

Table 6. Dimerization of (-)-**8** to **5**.[83]



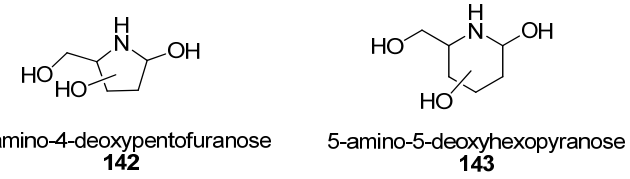
(-)-**8** (92% e.e.) | (-)-idesolide (**5**) | **140** | **141** (due to optical impurity in **8**)

Entry	Additive (equiv)	Temp	Time	**5** (%)	**140** (%)	**141** (%)
1	none	5 ˚C	48 h	0	0	0
2	NH_4Cl (1.0)	5 ˚C	20 h	0	0	0
3	$NaHCO_3$ (1.0)	5 ˚C	20 h	39	3	7
4	$NaHCO_3$ (1.0)	rt	20 h	51	5	8
5	$NaHCO_3$ (2.0)	rt	20 h	65	4	7

All of the syntheses presented suffered from non stereoselective transformations. Although good selectivity is achieved in the cases of Iwabuchi and Kuwahara, the enantiopure material is generated by chemical resolutions and separations. The syntheses of idesolide lack the benefit of an enantiopure raw material and instead rely on stereoselective chemical transformations.

2.3 Iminosugars

Iminosugars can be defined as sugars in which the endocyclic oxygen atom has been replaced by a basic nitrogen atom. This simple substitution has arguably formed the most attractive class of carbohydrate mimics reported and has raised many synthetic challenges in their preparation. Many names have been used to describe the iminosugar framework, such as azasugar and iminocyclitol. In the context of this document "iminosugar" will be used exclusively as "azasugar" and "iminocyclitol" are used in a technically incorrect manner to describe these hetero-sugar derivatives. The prefix *aza* is used when describing a molecule in which a carbon atom has been replaced with a nitrogen atom. The term "iminocyclitol" should be abandoned, as well, for it describes a completely different class of compounds, namely carbon-based polyhydroxylated compounds, *e.g.* inositols. Using the standard rules for carbohydrate nomenclature it is possible to name these nitrogen analogues of sugars without special descriptors, Figure 34.[104]



4-amino-4-deoxypentofuranose **142**

5-amino-5-deoxyhexopyranose **143**

Figure 34. Iminosugars and nomenclature.

From the point of view of synthesis, the history of iminosugars only dates back to the 1960's when several reports from different groups surfaced.[105-109] In 1966, Inouye *et al*. isolated nojirimycin (**144**) from bacteria (*Streptomyces*),[110] with a follow-up synthesis in 1968, [111] and indicated its antibiotic properties. Also in 1966, Paulsen published the first synthesis of 1-deoxynojirimycin (**145**).[112, 113] It wasn't until Bayer AG scientist

recognized the potent ability of 1-deoxynojirimycin to slow carbohydrate degradation by inhibition of α-glucosidases and, thus, become a viable anti-diabetic target, did the iminosugar surge take off.[114] Eventually a derivative of 1-deoxynojirimycin was developed and today is marketed as Glyset™ (**146**). Iminosugars are still very actively pursued as medicinal targets and several examples are represented in Figure 35. A categorical assessment based on ring – skeletal structure will be presented below with indication of biological significance.

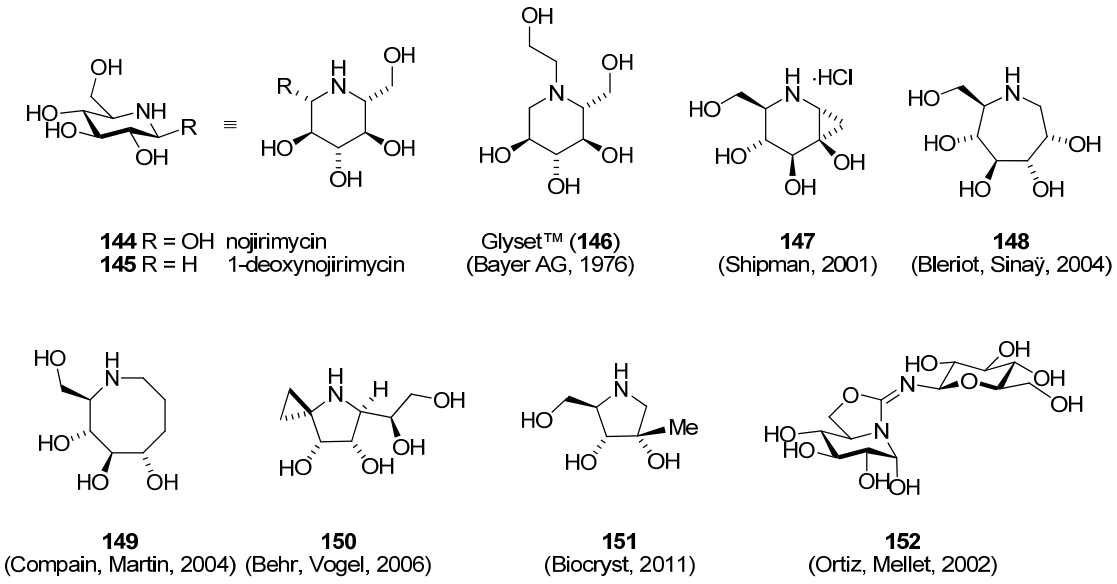


Figure 35. Iminosugar examples.[115-119]

2.3.1 Structural Diversity, Natural Sources, and Biological Activity

Piperidines

Nojirimycin **144** was the first naturally occurring iminosugar to be discovered and was isolated from the microorganism *Streptomyces roseochromogene* R-468.[120] Its common name was given after its isolation from *Streptomyces nojiriensis.*[121, 122] It had previously been ascertained that nojirimycin (**144**) had antimicrobial properties. It was also found to have potent inhibitory activity toward α- and β-glucosidases, as one might expect on the basis of structural similarity to glucose (**153**).[123] It was the initial discovery of nojirimycin and its biological activity that led to the findings of two other iminosugars from the culture broths of both species of *Streptomyces*: 5-amino-5-deoxy-D-manopyranose (nojirimycin B, **154**)[124] and 5-amino-5-deoxy-D-glactopyranose (galactostatin **155**), Figure 36.[125] Chemists interested in heterocyclic chemistry soon began isolating polyhydroxylated piperidine derivatives from plants. The first iminosugars to be isolated from a plant source was fagomine (**156**), from *Fagopyrum esculentum* (buckwheat),[126] and moranoline (a compound prepared up to this point synthetically, 1-deoxynojirimycin **145**), from a species of *Morus* (Mulberry trees).[127] Fagomine is also found in all parts of the mulberry tree cultivated in China, Korea, and Japan. The leaves of the mulberry tree have traditionally been used to cure and prevent "Xiao-ke" (diabetes) in Chinese herbal medicine. The root bark of the mulberry has also been used in Chinese herbal medicine for anti-inflammatory, diuretic, antitussive, and antipyretic purposes, while the fruits of the tree have been used as a tonic and sedative.[114]

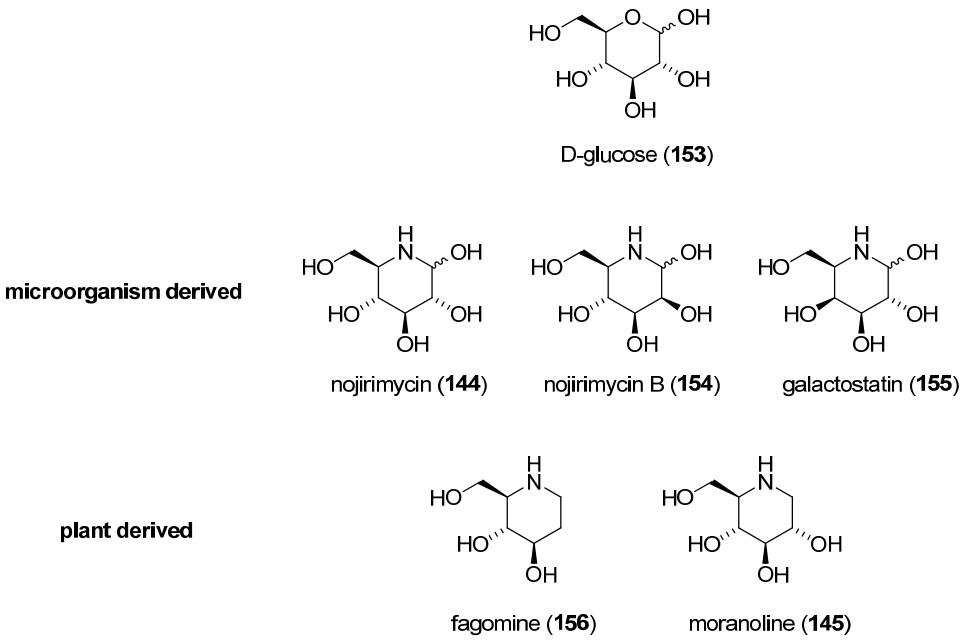


Figure 36. The first iminosugars isolated.

Pyrrolidines

Naturally occurring polyhydroxylated pyrrolidines first appeared in the scientific literature in 1976 with the isolation of 2,5-dihydromethyl-3,4-dihydroxypyrrolidine (DMDP, **157**) from species of the legume genus *Derris elliptica*.[128] The active pursuit of biologically active and naturally occurring pyrrolidine glycosidase inhibitors remained idle until DMDP (**157**) was re-isolated from the legume genus *Lonchocarpus*[129] and was shown to be have α- and β-glucosidase inhibition at 3.3 and 7.8 μM concentrations, respectively.[130] The absolute stereochemistry was not determined until 1987 when Fleet and coworkers report the enantiospecific synthesis, Figure 37.[131]

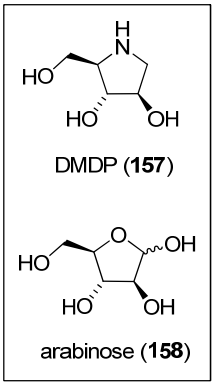


Figure 37. Natural DMDP.

Indolizidines

The first polyhydroxylated bicyclic alkaloid discovered was swainsonine (**159**) from the Australian legume *Swainsona canescens*[132] and was followed closely with the report of the more heavily oxidized castanospermine (**160**).[133] Swainsonine has been shown to be an inhibitor of Golgi α-mannosidse II, an enzyme involved in the N-linked polysaccharide (glycan) processing and has shown some possibilities toward chemotherapeutic therapies by means of anti-metastatic immunomodulation of melanoma cells.[134] The biosynthesis has been investigated in the fungus *Rhizoctonia leguminicola* and involves the initial conversion of lysine (**161**) to pipecolic acid (**162**), Figure 38.[135] The pyrrolidine portion of swainsonine is constructed by the contribution of two carbons atoms from acetate that eventually forms 1-oxoindolizidine **163**. A reduction of **163** occurs followed by hydroxylation at C-2 to give **165**. An unusual epimerization of **165** then occurs with subsequent hydroxylation to yield swainsonine (**159**).

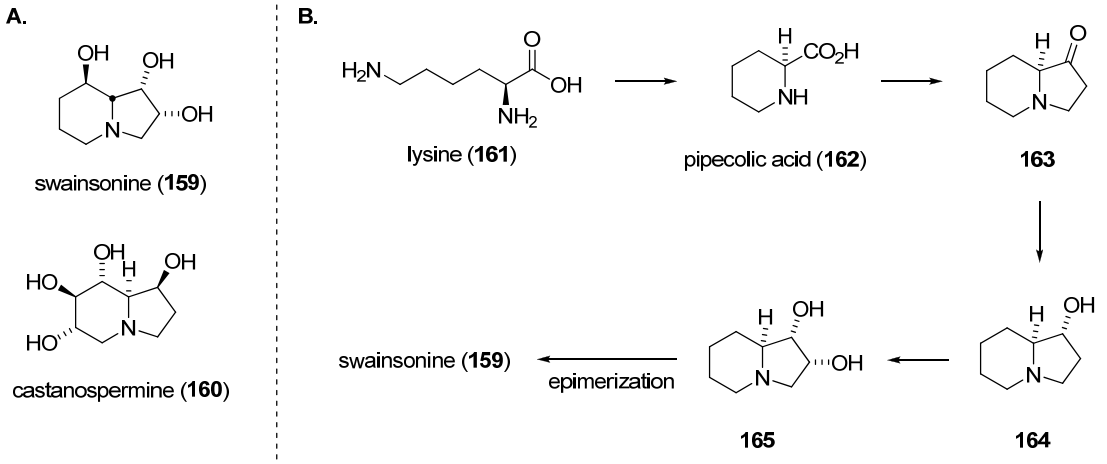


Figure 38. (A) Natural indolizidines. (B) Proposed biosynthetic pathways for swainsonine.

Castanospermine, on the other hand, has been shown to be biologically active as a potent glucosidase inhibitor and antiviral agent. Castanospermine inhibits lysosomal α-

glucosidase and disturbs the lysosomal catabolism of glycogen, thus, causing the accumulation of glycogen in the lysosome leading to muscle and nerve cell damage (Pompe disease).[136] The castanospermine containing legume *Castanospermum austral* was recognized as the toxic source for livestock, leading to the Pompe-like symptoms, which led to its initial isolation. The antiviral activity exhibited by castanospermine comes as a result of the disruption of structural protein folding that is critical to viral secretion. These findings were substantiated in both *in vitro* and *in vivo* studies.[137]

Pyrrolizidines

Australine (**166**)[138] and alexine (**167**)[139] were the first two polyhydroxypyrrolizidines (containing more than two hydroxyl groups) discovered and were both reported in 1988, Figure 39. The species *Castanospermum austral* is native to the northeastern part of Australia and has been introduced into the Indian subcontinent, South Africa, and mild climate areas of North America as ornamental trees. Castanospermine (**160**) is the major component of the chestnut-like seed that australine (**166**) was isolated from. Identification and absolute stereochemical assignment for **166** was accomplished by NMR, mass spectrometry, and X-ray crystallography. It was determined that australine is a potent and specific inhibitor of α-glucosidase (5.2 μM).

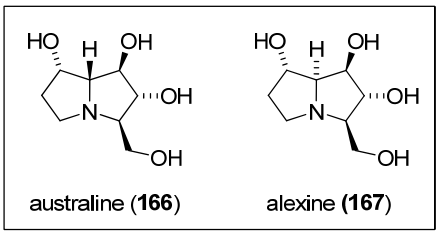


Figure 39. First isolated pyrrolizidines.

Alexine (**167**) is epimeric to australine (**166**) at the bridgehead position and is isolated from *Alexa leiopetala*, trees native to the wet lands of Guyana, Surinam,

Venezuela, and the Amazon basin. The structure of alexine was assigned by NMR and X-ray crystallography. The biological activity of this alkaloid was less impressive in comparison to to the epimer **167** and showed a weak inhibition of β-glucosidase and β-galactosidase.

*Nor*tropanes

The most recently recognized members of the iminosugar group are the tri-, tetra-, and pentahydroxy *nor*tropane alkaloids, the calystegines **168** – **177**, and are differentiated from the tropane alkaloids by the lack of *N*-methyl substituent and the presence of hydroxyl group at the bicyclic ring bridgehead, Figure 40. These alkaloids were isolated from the roots of *Calystegia sepium* in efforts to identify substances with selective effects on plant – microbial relationship.[140] It was found that the calystegines, released from the roots of *C. sepium*, acted as nutritional mediators for microbial growth. The study identified the plasmid of *Rhizobium meliloti* that encodes for catabolism of these uniquely structured alkaloids. No biological activity has been reported for this series of natural products, only catabolism studies relating to soil bacteria.

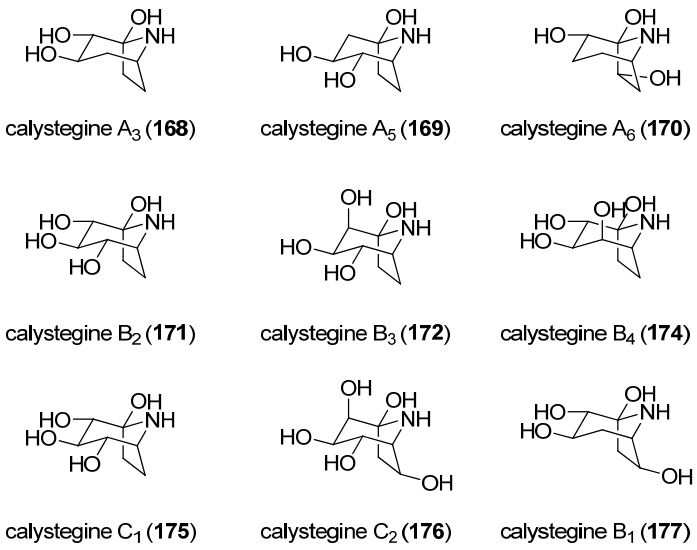


Figure 40. *Nor*tropane alkaloids (calystegines).

Glycosides

Of the five classes of alkaloids presented above four are known to exist as glycosides. Indolizidines are the only class not known to form glycosides. Piperidine glycosides are by far the most numerous. The glycoside of fagomine was isolated early on from the legume of *Xanthocercis zambesiaca*.[141] Other glycosides have remained undetected until recently, when technological advances made the isolation of such species more achievable. Known iminosugar-producing natural sources have thus been re-examined to identify other glycoside derivatives. The renewed interest in the identification of the glycosidic derivatives of iminosugars has been successful in the identification of seven glucosides and two galactosides of 1-deoxynojirimycin (**145**) from the species of *Morus*.[142] Only one example is known of a polyhydroxylated pyrrolizidine, belonging in the *nor*tropane class, glucosides of casuarine[143] and calystegine B_1 (**177**)[144] respectively.

The wide distribution of iminosugar-producing plants dictates the large variation of these alkaloids observed from natural sources.[145] Several plant families have been identified that produce such chemical species and include Moraceae, Euphorbiaceae, Leguminosae, Campanulaceae, Polygonaceae, Myrtaceae, Araceae, Hyacinthaceae, Casuarinaceae, Convolvulaceae, and Solanaceae.[146] Equally as distributed is the range of biological activities that iminosugars exhibit and can be summarized by a few enzymatic processes that include glycosidases, glycosyltransferases, nucleoside-processing enzymes, glycogen phosphorylases, and metalloproteinases, Table 7.[147] The therapies that are targeted are just as broad in scope and are also depicted below.

Table 7. Enzyme and therapeutic targets.

Enzymatic targets	Therapeutic targets
Glycosidases (mid-1970's)	Diabetes (mid-1970's)
Glycosyltransferases (1992)	Viral diseases (1980's)
Nucleoside-processing enzymes (1993)	Cancers (1980's)
UDP galactopyranose mutase (1997)	Lysosomal diseases (1990's)
Glycogen phosphorylaes (1997)	Psoriasis (2000's)
Metalloproteinases (2004)	Cystic fibrosis (2006)

2.3.2 Selected Syntheses and General Strategies

The vast amount of iminosugar derivatives, their skeletal features, and the various ring sizes make any strategic synthetic analysis difficult. As a consequence, this section will focus on the synthetic strategies focused at the formation of pyrrolidine based iminosugars that contain a tertiary alcohol at C-2 (carbohydrate numbering). Many reviews have been published that cover pyrrolidine and iminosugar construction strategies.[147-151]

Bols (1996)

In 1996 Bols and coworkers set forth the hypothesis that if a predictable transition state analogue could be constructed, specific glycoside hydrolases could be inhibited by structural modifications – certainly not a new concept by any means (structure-activity relationship).[152] However, no structural modification in the sense of a pyrrolidine analogue had been reported for the powerful glucoside hydrolase (β-glucosidase, K_i = 110 nM) isofagomine (**178**).[153] By doing so they hypothesized that a pyrrolidine analogue could selectively inhibit a pentofuranoside cleaving enzyme, such as purine nucleoside phosphorylase (PNP), over other pyranoside glycosidases. PNP catalyzes the conversion of ribonucleotides to purine and α-D-ribofuranose-1-phosphate **179** through a ribofuranosyl cation **180**, Figure 41. The iminosugar moiety is thought to inhibit glycosidases *via* amine protonation such as depicted in **181**, protonated isofagomine, or as an example of a more structurally similar derivative the ribofuranosyl analogue **182**.

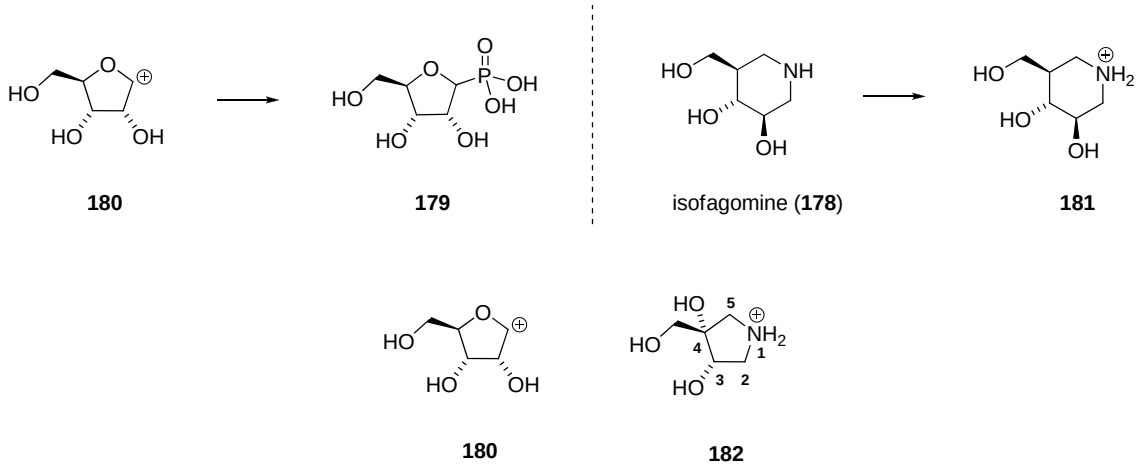
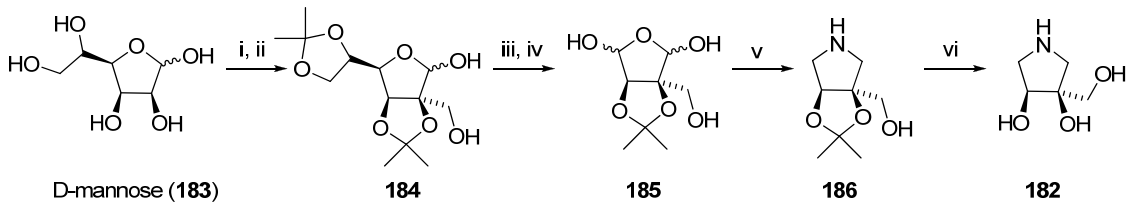


Figure 41. Transition state mimics – isofagomine **181** and *ribo*-isofagomine **182**.

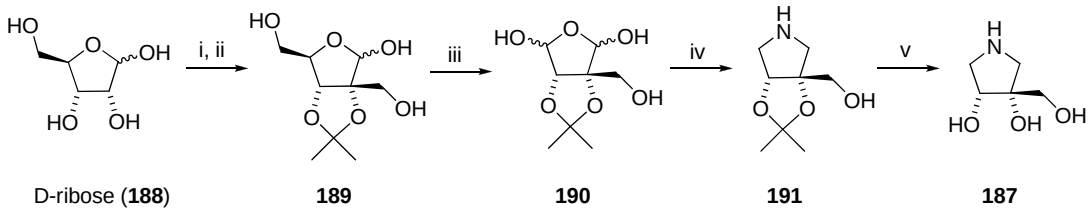
To test this hypothesis Bols and coworkers prepared a pyrrolidine analogue **182** that contained an additional hydroxy group at the 4-position in the belief that the 4-OH was less important for biological activity and because both enantiomers could be prepared more readily from the chiral pool. Pyrrolidine **182** was thus prepared from D-mannose (**183**) using Ho's aldolcondensation procedure[154, 155] that installed the quaternary carbon, *via* hydroxymethylation and stereo definition, Scheme 5. Ho's procedure consisted of bis isopropylidene formation and treatment with potassium carbonate (K_2CO_3) to form the hydroxymethylated intermediate **184**. The more sterically exposed acetonide in **184** was selectively hydrolyzed and the resulting vicinal diols were oxidatively cleaved with sodium periodate ($NaIO_4$) to give aldehyde-hydrate **185**. The amination of **185** to yield **186** was performed under stringent reaction conditions in order to avoid competitive reaction pathways (high pressure H_2 and low concentrations of NH_3). Subsequently, the acetonide in **186** was hydrolyzed under acidic conditions to yield iminosugar **182**.



Reagents: (i) 2,2-dimethoxypropane, acid catalyst (yield not reported); (ii) K_2CO_3, HCOH (37% aq), MeOH, 85 °C, 48 h (86%); (iii) H_2SO_4 (cat), MeOH (20% aq) (98%); (iv) $NaIO_4$, H_2O (86%); (v) NH_4OH (0.25 M), Pd/C, MeOH, H_2 (37 atm) (87%); (vi) HCl, 55 °C (65%).

Scheme 5. Synthesis of iminosugar **182**.

Alternatively, the synthesis of enantiomer **187** proceeded from D-ribose (**188**) by an acetonation by a method first reported by Hughes and Speakman[156] and subsequent Ho hydroxymethylation[154, 155] to yield **189**. For iminosugar **187** chemistry analogous to that performed in the preparation of **182** was used, followed by the oxidative cleavage of a "masked" vicinal diol of **189** using $NaIO_4$ to produce aldehyde-hydrate **190**, amination to give acetonide protected pyrrolidine **191**, and acid hydrolysis to provide **187**, Scheme 6.



Reagents: (i) H_2SO_4, acetone (59%); (ii) K_2CO_3, HCOH (37% aq), MeOH, 85 °C (yield not reported); (iii) $NaIO_4$, H_2O, 25 °C, 18 h (94%); (iv) NH_4OH (0.25 M), Pd/C (5%), H_2 (102 atm), EtOH (92%); (v) HCl (4 M aq), 25 °C, 18 h (51%).

Scheme 6. Synthesis of iminosugar **187**.

Bols and coworkers prepared a set of enantiomerically pure, polyhydroxylated, pyrrolidines **182** and **187** by a series of transformations that included a stereospecific aldol condensation to install the quaternary chiral center at C-2 (sugar numbering) that, if not for the anomeric nature of the sugar derivative and the *cis*-diol functionality that was

sequestered as an acetonide, would have been difficult to prepare. The iminosugars were tested for inhibition of α-glucosidase from bakers yeast and β-glucosidase from almonds but showed no significant activity and were non-specific.

Fleet (2010)

Pyrrolidine **192** was isolated from *Arachniodes standishii* and *Angylocalyx boutiqueanus* in 1985[157-159] and showed strong inhibition of α-glucosidase and weaker inhibition of other glucosidases. The unnatural enantiomer **193** was prepared and demonstrated stronger and more selective α-glucosidase inhibition than **192**, as often the unnatural iminosugar isomers have been shown to do.[160, 161] Analogues of both iminosugar enantiomers **192** and **193** were prepared as iminosugars **194** and **195** from the chiral pool, D-ribose (**188**) and D-tagatose (**196**), respectively. Biological studies indicated that **194** was active in glucosidase inhibition, the first example of a branched iminosugar pyrrolidine showing significant glucosidase activity, Figure 42.[162]

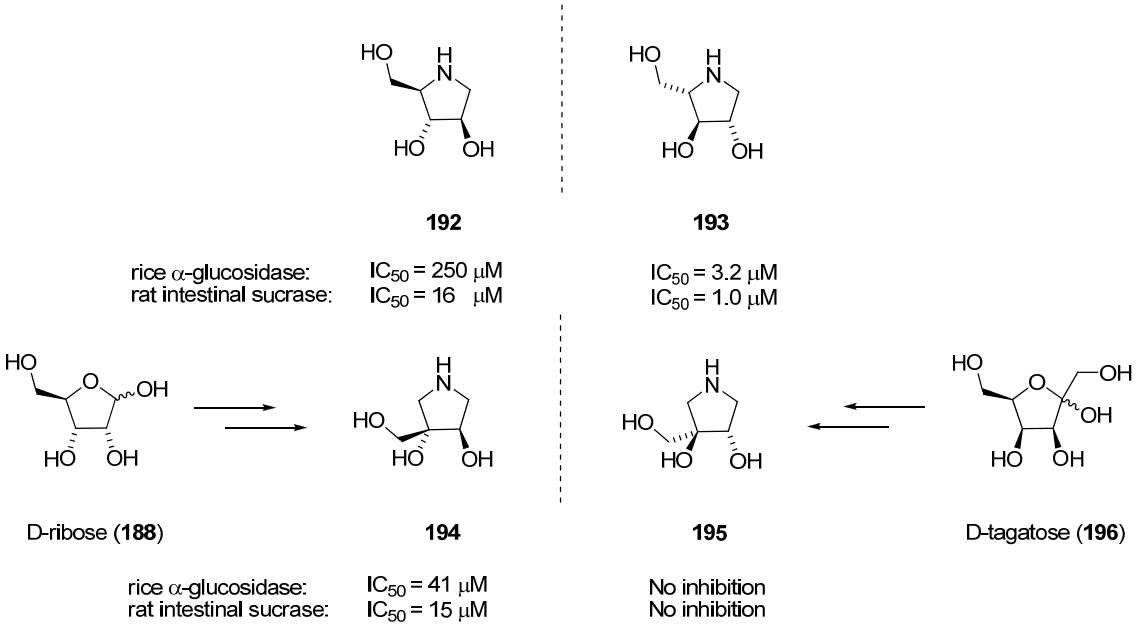
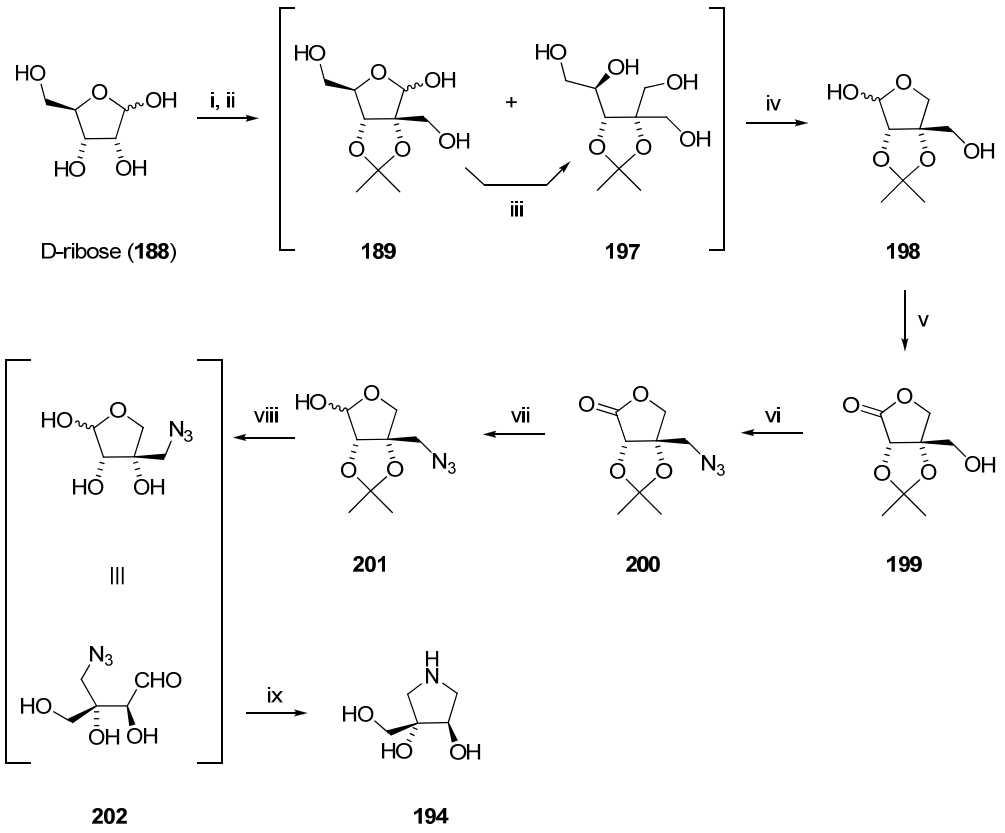


Figure 42. Synthesis of natural and unnatural iminosugars **194** and **195**.

The synthesis of **194** is an eight step preparation from D-ribose consisting of only a single use of a protecting group, and proceeding with an overall yield of 37%, Scheme 7. Fleet and coworkers utilized Ho's hydroxymethylation procedure[154, 155] that provided a mixture of hemiacetal **189** and tetrol **197** that was treated with $NaBH_4$ to effect full reduction of the mixture to **197**. Oxidative cleavage of **197** to **198** followed by oxidation with Br_2/H_2O, activation of the primary alcohol and subsequent displacement of the mesylate with azide provided azide **200**. The final amination sequence started by re-forming the hemiacetal *via* reduction of γ-lactone **200** with diisobutylaluminum hydride (DIBAL-H) yielding **201**, hydrolysis of the isopropylidine in **201** with Dowex resin, and reduction of the azide with concomitant cyclization of the amine *via* condensation yielded iminosugar **194**.

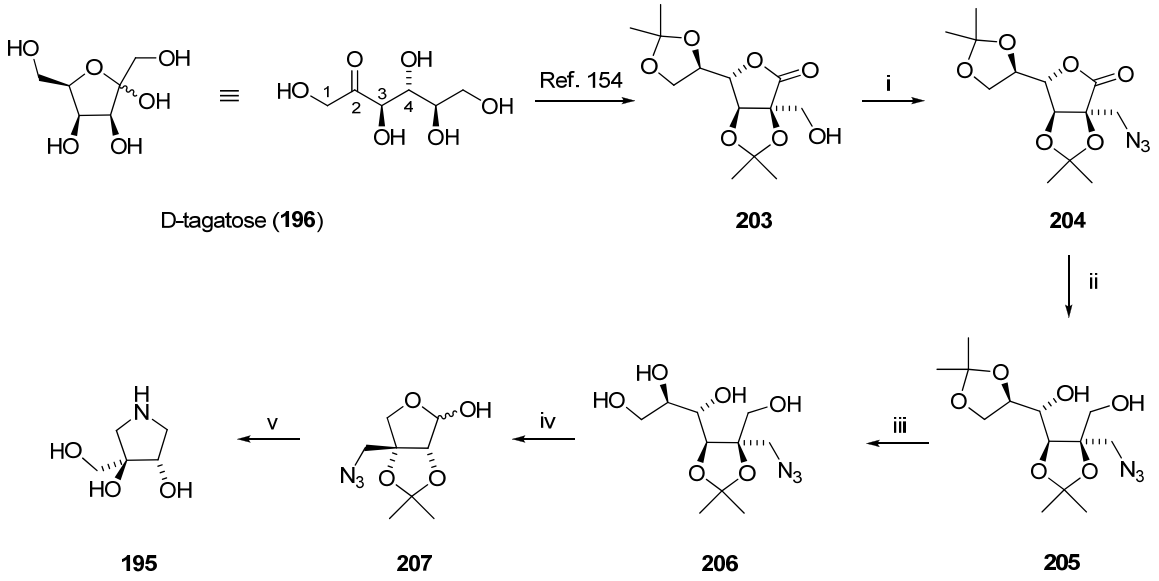


Reagents: (i) H_2SO_4, acetone (59%); (ii) CH_2O, K_2CO_3, MeOH, H_2O; (iii) $NaBH_4$, H_2O; (iv) $NaIO_4$, H_2O (84% from acetonide, not shown); (v) Br_2, $BaCO_3$, H_2O, 0 °C → rt (90%); (vi) $(CF_3SO_2)_2O$, py, CH_2Cl_2, -30 °C; then NaN_3, DMF (67%); (vii) DIBAL-H, CH_2Cl_2, -78 °C (93%); (viii) Dowex (50 W-X_8 H^+ form) H_2O/1,4-dioxane (4:1), 75 °C (100%); (ix) Pd/C (10%), H_2 (1 atm), H_2O/HOAc (9:1) (71%).

Scheme 7. Fleet's synthesis of iminosugar **194**.

Compound **195**, the enantiomer of **194**, was prepared from D-tagatose (**196**) and the synthesis was started with a Kiliani-Fischer reaction to introduce the C-2 hydroxymethyl group *via* treatment of tagatose with sodium cyanide (NaCN) in water followed by acid hydrolysis and acetonation to yield diacetonide **203**,[163] a common chiron used in the field of sugar chemistry. The activation of primary alcohol **203** with triflic anhydride (Tf_2O), displacement of the triflate group with azide, and the two step reduction with DIBAL-H/$NaBH_4$ afforded azido diol **205**. Sodium periodate was used to oxidatively cleave the C-4 and C-5 bonds which allowed for cyclization to occur and

yield azido pentose **207**. The final cyclization to pyrrolidine **195** was performed as a one-pot procedure, in which acid hydrolysis of **207** cleaved the isopropylidene to yield the *vic* diol (not shown). The reaction mixture was then treated with palladium/charcoal (Pd/C) under an atmosphere of hydrogen in order to reduce the azido group to an amine, which underwent spontaneous cyclization to pyrrolidine **195**. The practical and simple procedure led to the preparation of polyhydroxylated pyrrolidine **195**, achieved in 47% overall yield from **203**.



Reagents: (i) Tf_2O, py, CH_2Cl_2, -30 °C; then NaN_3, DMF (94%); (ii) DIBAL-H, CH_2Cl_2, -78 °C; then $NaBH_4$, MeOH (90%); (iii) HOAc/H_2O (1:1) (83%); (iv) $NaIO_4$, H_2O (90%); (v) Dowex (50 W-X8 H^+ form), H_2O/1,4-dioxane (4:1), 75 °C; then Pd/C (10%), H_2 (1 atm), H_2O/HOAc (9:1) (74%).

Scheme 8. Fleet's synthesis of enantiomer **194**.

Kotian (2010)

In 2010 BioCryst Pharmaceuticals, Inc. reported two syntheses of iminosugar **151**. Two other iminosugars, **208** and **209**, have been previously reported by BioCryst Pharmaceuticals and were used in the preparation of compounds **210** and **211**,

respectively, in their effort to identify potent inhibitors of human purine nucleoside phosphorylase (PNP), Figure 43.[119]

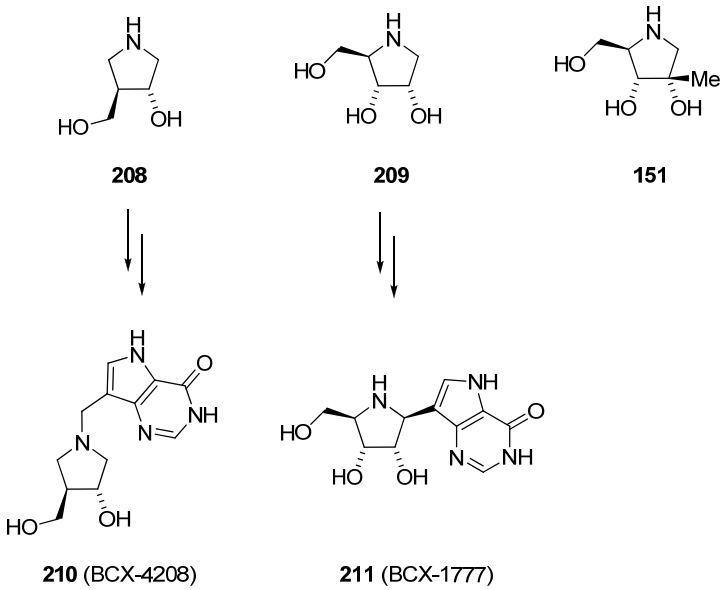
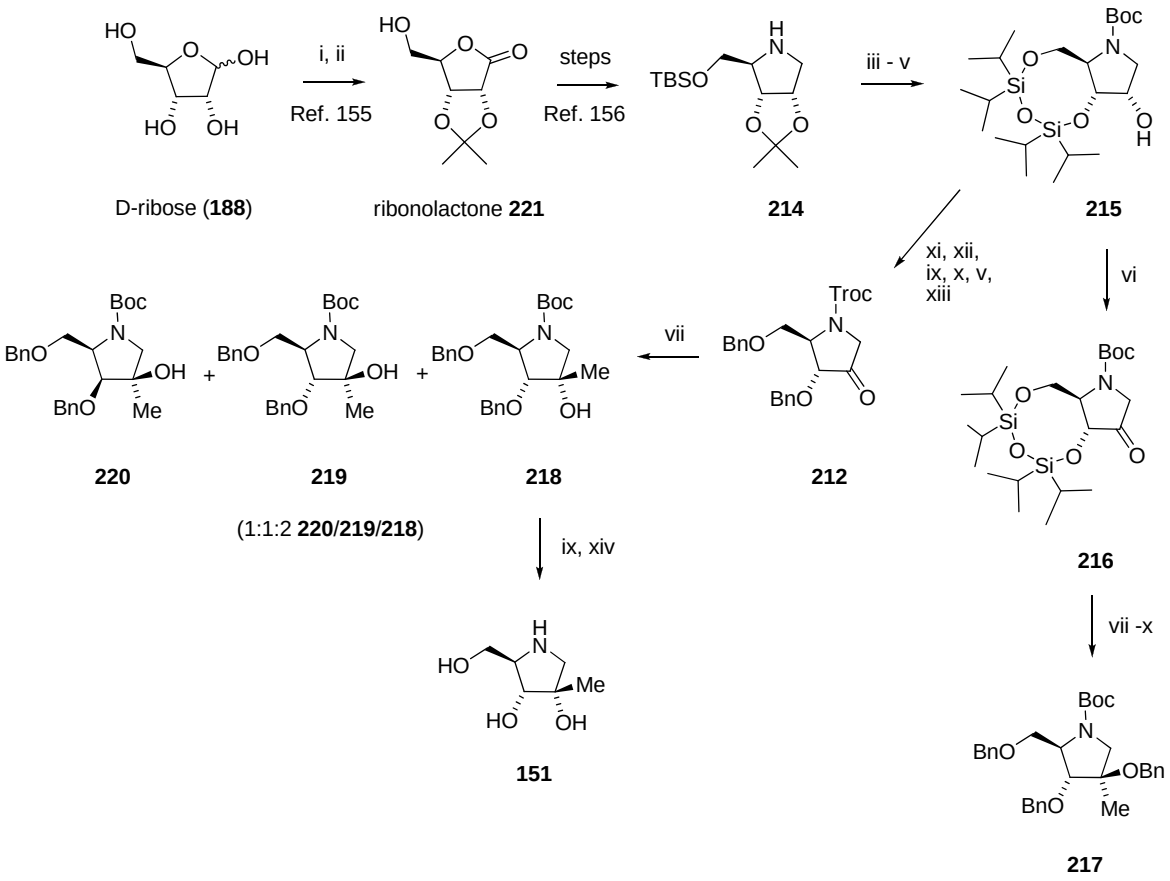


Figure 43. BioCryst Pharmaceuticals PNP inhibitors.

The strategy that was employed for the synthesis of **151** is unique for this class of quaternary center–containing pyrrolidines in that the establishment of the quaternary center was performed by a stereoselective addition of methyl magnesium bromide to ketone **212** (prepared from D-ribose), Scheme 9, and, alternatively, through stereoinduction derived from protected D-serine derivative **213**, Scheme 10.

The first approach to **151** started from protected pyrrolidine **214**, derived from D-ribose (**188**) in several steps,[164, 165] and involved multiple protecting group manipulations to single out the secondary hydroxyl group in **215** for oxidation to **212** and **216**, Scheme 9.[166] The initial attempts at a stereoselective addition of methyl Grignard to the β-face of **216** was mediated by titanium(IV) chloride ($TiCl_4$) and found to be selective toward α-addition, presumably due to nucleophilic approach from outside the concave face of

bicycle **216**. The addition of methyl magnesium bromide to ketone **212** was only slightly more selective towards β-addition and gave **218** as the major component in a mixture of products that also contained **219** and **220** (**218**/**219**/**220** 2:1:1). The benzylation of the mixture containing **218** followed by separation and subsequent global deprotection yielded pyrrolidine **151**. The pitfall of this particular route was the loss of stereo control during the $TiCl_4$–mediated addition of methyl magnesium bromide to ketone **212** and prompted an alternative approach to **151**.

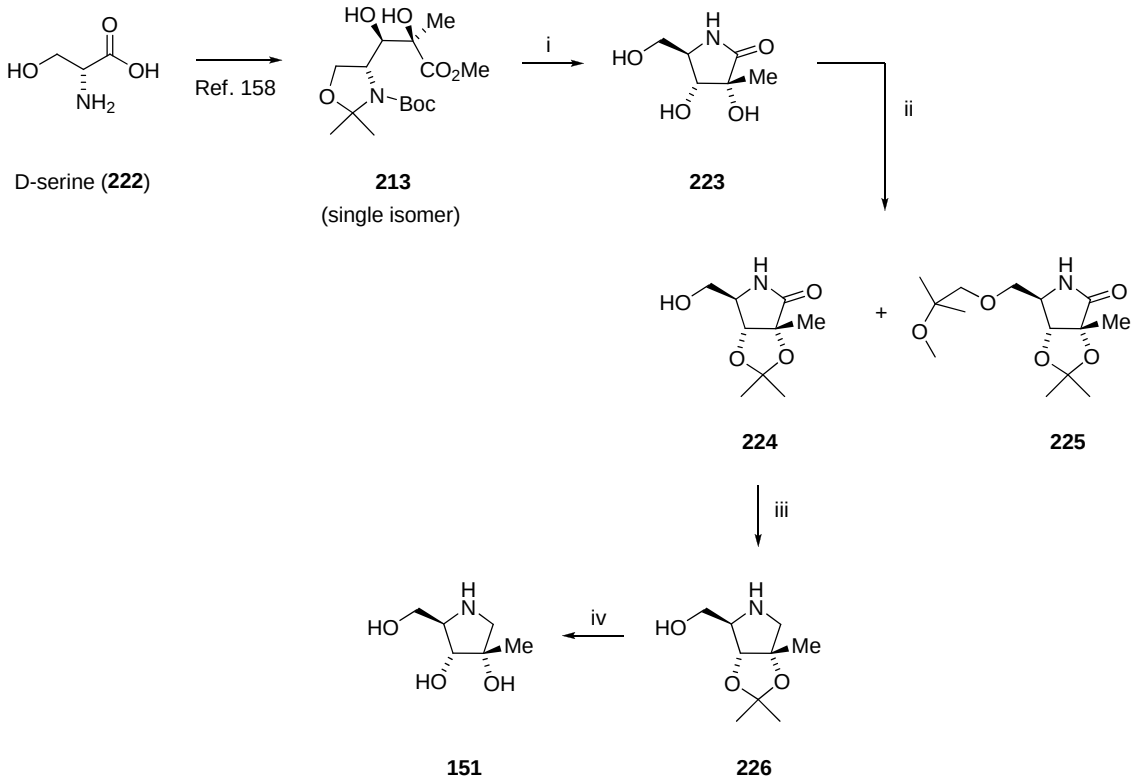


Reagents: (i) Br_2, $NaHCO_3$, H_2O; (ii) 2,2-DMP, Ag_2CO_3, H_2SO_4, acetone (73% from **188**); (iii) TFA/ H_2O (10% v/v); (iv) Et_3N, Boc_2O; (v) 1,3-dichlorotetraisopropyldisiloxane; (vi) CrO_3; (vii) $TiCl_4$, MeMgBr; (viii) TBAF; (ix) NaH, BnBr; (x) TFA, THF, H_2O; (xi) MOMBr, DIPEA; (xii) TBAF, THF, MeOH; (xiii) DMSO, TFAA, Et_3N; (xiv) HCl, Pd/C, MeOH.

Scheme 9. Synthesis of pyrrolidine **151** from D-ribose.

The second approach reported by Kotian *et al*. utilized a derivatized D-serine derivative **213**[167] to establish the stereochemical control needed to generate enantiomerically pure polyhydroxylate lactam **223**. The isopropylidine derivatives **224** and **225** were separated and intermediate **224** was reduced with lithium aluminum hydride ($LiAlH_4$) to pyrrolidine **226**. Acid hydrolysis of **226** furnished iminosugar **151**.

This synthetic route proved to be more direct and was less reliant on protecting group strategies than the first synthetic route, Scheme 9.



D-serine (**222**) **213** (single isomer) **223** **224** **225** **151** **226**

Reagents: (i) HCl, MeOH, base, pH 7; (ii) 2,2-DMP, DMF, *p*-TsOH (cat); (iii) LiAlH4; (iv) HCl/MeOH.

Scheme 10. Synthesis of pyrrolidine **151** from D-serine.

The field of sugar chemistry has a long history. The duration at which the field of study has had to mature has enabled the development of many creative and practical methods for the synthesis of sugars and sugar-like compounds. Generally, most of the synthetic endeavors toward these kinds of structures take advantage of the chiral pool as raw materials and rely on the manipulation of functionalities for both chemo- and stereo-induced transformations.

2.4 Vinca and Aspidosperma Alkaloids

The terpene indole alkaloids constitute a diverse class of natural products that contains over 2000 members.[168] A range of diverse biological activities is exhibited by this class of alkaloids and includes anti-cancer, anti-malarial, and anti-arrhythmic activities. Relatively few reports of the isolation of new monoterpenoid alkaloids are seen each year. The focus of modern research activity for these natural products is in the field of synthetic organic chemistry as the biosynthetic pathways have been well established. The structural complexity of the monoterpenoid indole alkaloids is such that it has provided a realm of new synthetic strategies and the development of chemical methods. Numerous research programs have been aimed at tackling the challenge of an efficient synthetic strategy and, more academically, devising methodologies for regio-, enantio-, and chemoselectivity.

Vindoline (**7**), a major alkaloidal constituent of *Cantharanthus roseus* (*C. roseus*), represents the lower portion of vinblastine (**13**) and serves as its biosynthetic precursor, Figure 44. Vinblastine (**13**) and vincristine (**14**) are the most widely recognized members of the vinca alkaloids because of their clinical use as antitumor drugs (Velban®/Velbe® and Oncovin®, respectively). Other vinca alkaloids that have been investigated include desoxyvincaminol **227**, vincaminol **228**, and vinburnine **229**. Semisynthetic vinca derivatives have been developed as antitumor drugs, such as Eldesin® (**230**),[169] Navelbine® (**231**),[170, 171] and Javlor® (**232**),[172, 173] and contain a contracted velbanamine (**233**) upper subunit compared to the parent natural products. Vincristine (**14**) was one of the first natural products whose structural elucidation was done by x-ray crystallography

and, also, among the first compounds in which a heavy atom derivative was used to determine the absolute configuration.[174]

In the following sections biosynthetic consideration will be depicted for this class of alkaloids with emphasis on biological activities and general strategies toward the synthesis of vindoline (**7**) and closely related natural products. The large literature compilation of monoterpenoid indole alkaloids research prevents a comprehensive analysis of the entire field. As a result, a limited survey of vinca alkaloids, namely vindoline (**7**), will follow with a mention of related synthetic strategies to other aspidosperma and vinca alkaloids.

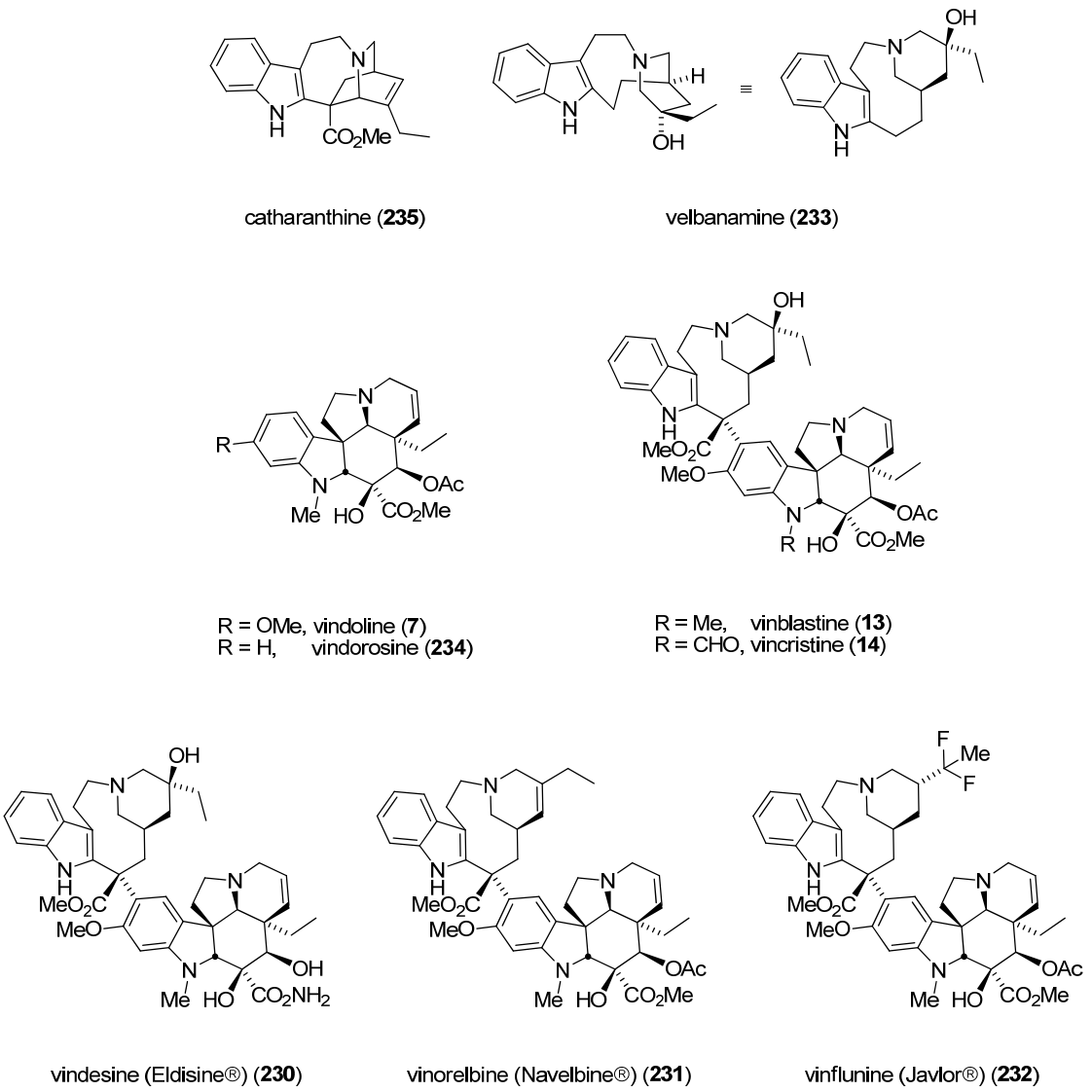


Figure 44. Natural vinca alkaloids and unnatural derivatives.

2.4.1 Biosynthesis of Aspidosperma – Vinca Alkaloids

It is well understood that the biosynthesis of many monoterpene indole alkaloids originates in the condensation of the iridoid terpene secologanin (**236**) and tryptamine (**237**) to form strictosidine (**238**) *via* strictosidine synthase, Figure 45. Tryptophan (**239**) undergoes decarboxylation to form tryptamine (**237**) through tryptophan

decarboxylase.[175] The enzyme tryptophan decarboxylase has been extensively studied, isolated and its cDNA sequence cloned.[176]

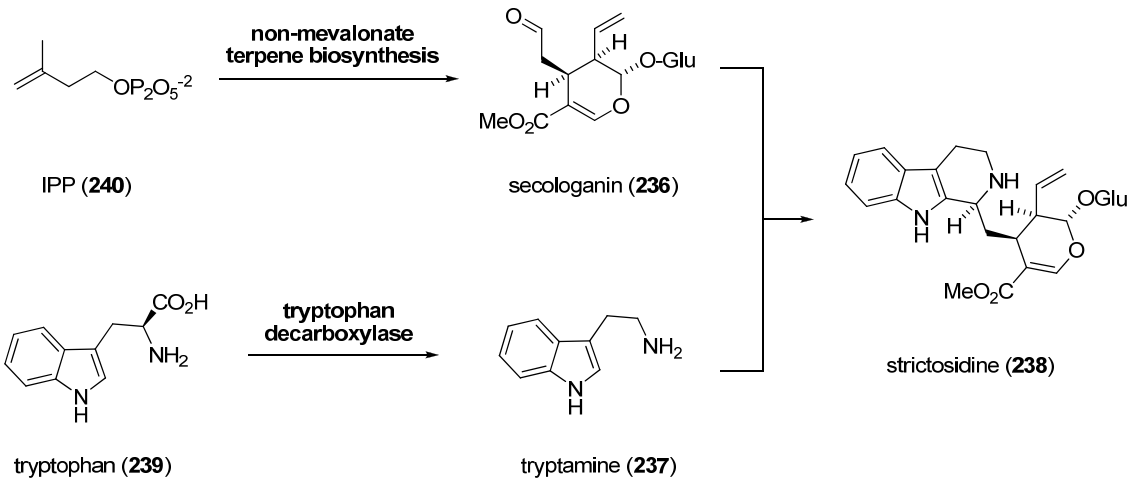
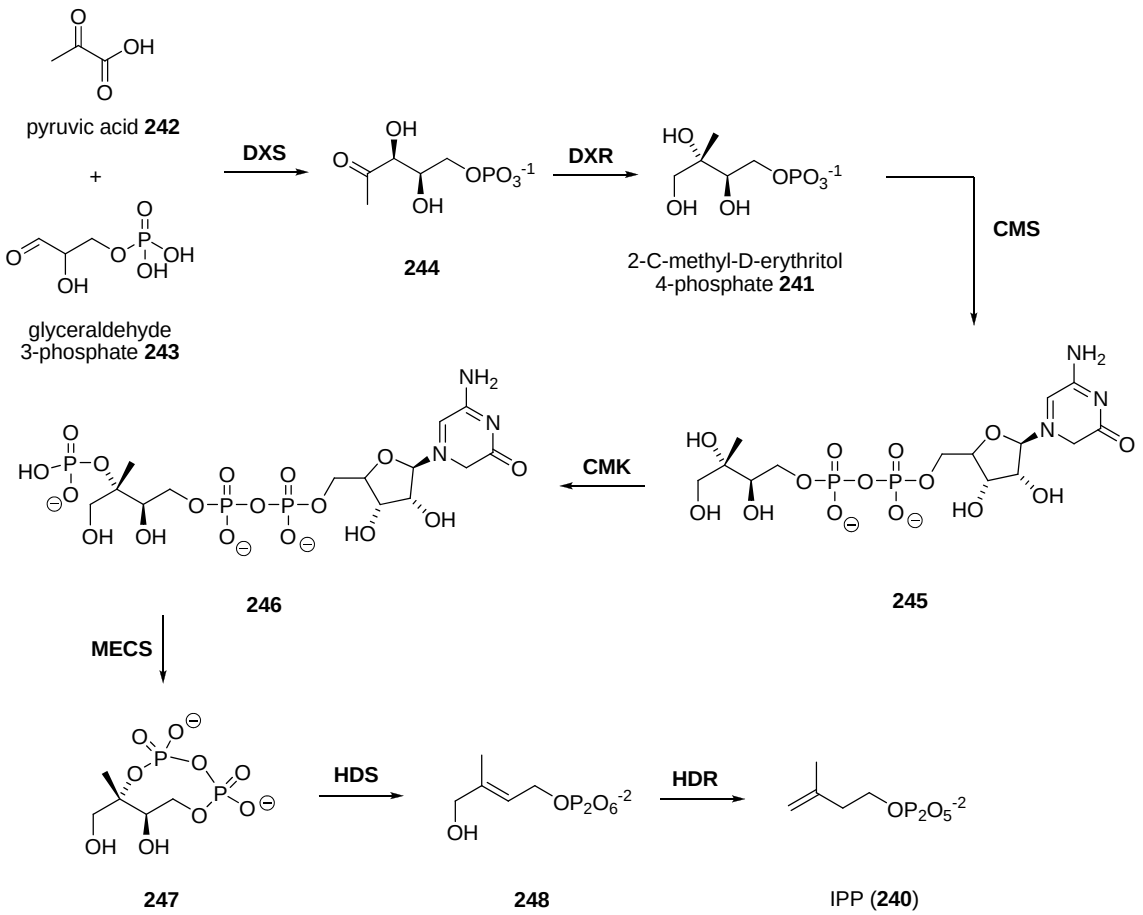


Figure 45. Biosynthetic Pictet-Spengler reaction of secologanin and trypamine.

Until recently, the classical mevalonic acid (MVA) pathway was thought to be the only biosynthetic process and, thus, the source of isoprene diphosphate (IPP) (**240**). It is now well understood and proven that plants, periwinkle in particular, use a mevalonate–independent pathway to produce IPP (**240**) that was first identified in bacteria and is called the 2-*C*-methyl-D-erythritol 4-phosphate (MEP) **241** pathway.[177-179] Pyruvic acid **242** and glyceraldehyde 3-phosphate **243** initiate the sequence leading to IPP (**240**) and all enzymes of the MEP pathway have been encoded by single genes with the exception of one, 1-deoxy-D-xylulose 5-phophate synthase (DXS), Scheme 11.[180]

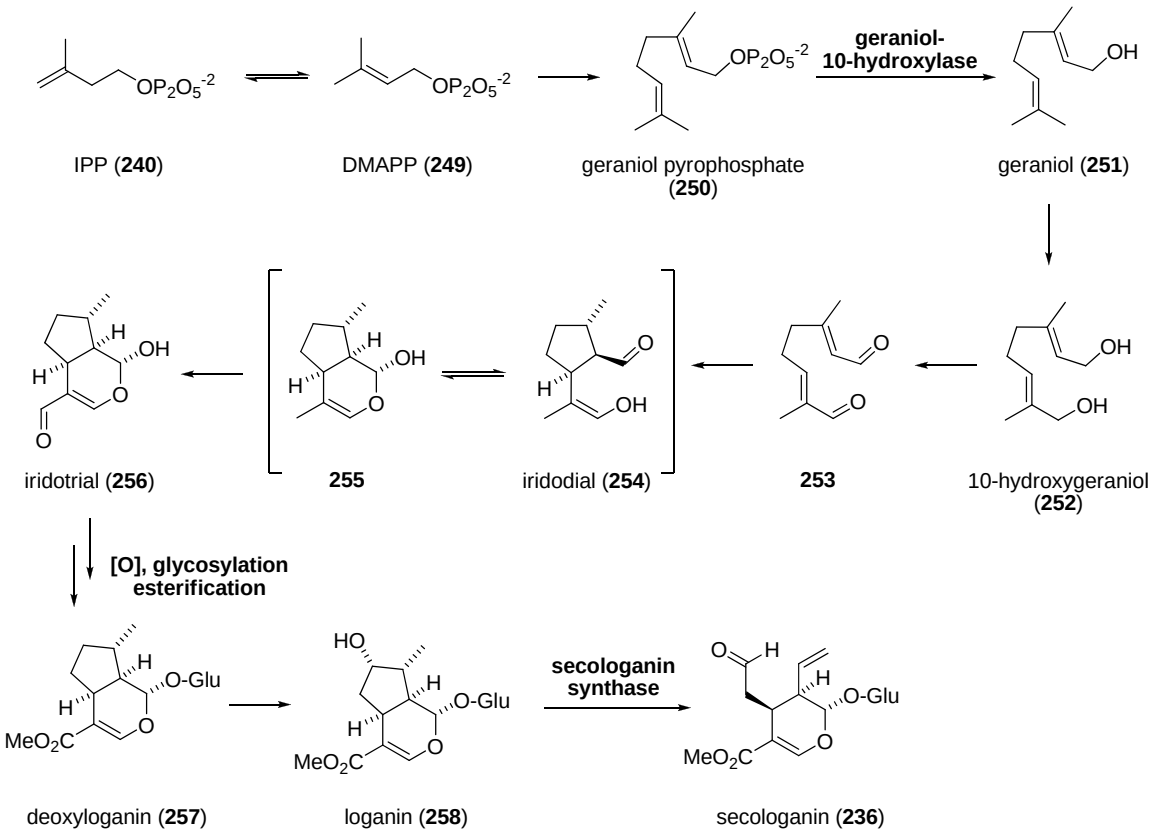


Abbreviations: DXS: 1-deoxy-D-xylulose 5-phosphate synthase; DXR: 1-deoxy-D-xylulose 5-phosphate reductase; CMS: 4-diphosphocytidyl-2-*C*-methyl-D-erythritol synthase; CMK: 4-diphosphocytidyl-2-*C*-methyl-D-erythritol kinase; MECS: 2-*C*-methyl-D-erythritol 2,4-cyclodiphosphate synthase; HDS: 1-hydroxy-2-methyl-2-butenyl 4-diphosphate synthase; HDR: 1-hdyroxy-2-methyl-2-butenyl 4-diphosphate reductase.

Scheme 11. MEP pathway for the biosynthesis of IPP.

The biosynthesis of secologanin (**236**) continues upon coupling of IPP (**240**) and dimethylallyl pyrophosphate (**249**) to form the monoterpene geraniol pyrophosphate (**250**). The first committed step toward an iridoid skeleton is the hydroxylation of geraniol (**251**) to 10-hydroxygeraniol (**252**) by geraniol-10-hydroxylase (G10H), Scheme 12. Geraniol-10-hydroxylase has been expressed in yeast and was shown to hydroxylate geraniol (**251**) *in vitro*.[181, 182] ^{3}H-labeled terpene experiments performed by Uesato *et al*.

suggest that 10-hydroxygeraniol (**252**), iriodial (**254**), and iridotrial (**256**) are all intermediates in biosynthesis of secologanin (**236**).[183, 184]



Scheme 12. Continuation of the non-mevalonate biosynthesis of secologanin from IPP.

Nearly all of the enzymes involved in the MVA, MEP and iridoid pathways in *C. roseus* have been characterized.[180] In plants, the MEP pathway is responsible for the generation of monoterpenes, diterpenes, and the prenyl side chains of chlorophylls and carotenoids. Once secologanin (**236**) is formed strictosidine synthase catalyzes the condensation of tryptamine (**237**) and secologanin (**236**) to generate strictosidine (**238**) *via* a stereoselective Pictet-Spengler cyclization, Figure 45. The formation of strictosidine (**238**) provides a biosynthetic intermediate that leads to several diverse classes of terpene indole alkaloids, Figure 46. Strictosidine synthase was first isolated, cloned, and its substrate specificity examined by Kutchan *et al*. in the late 1980's and early 1990's.[185, 186]

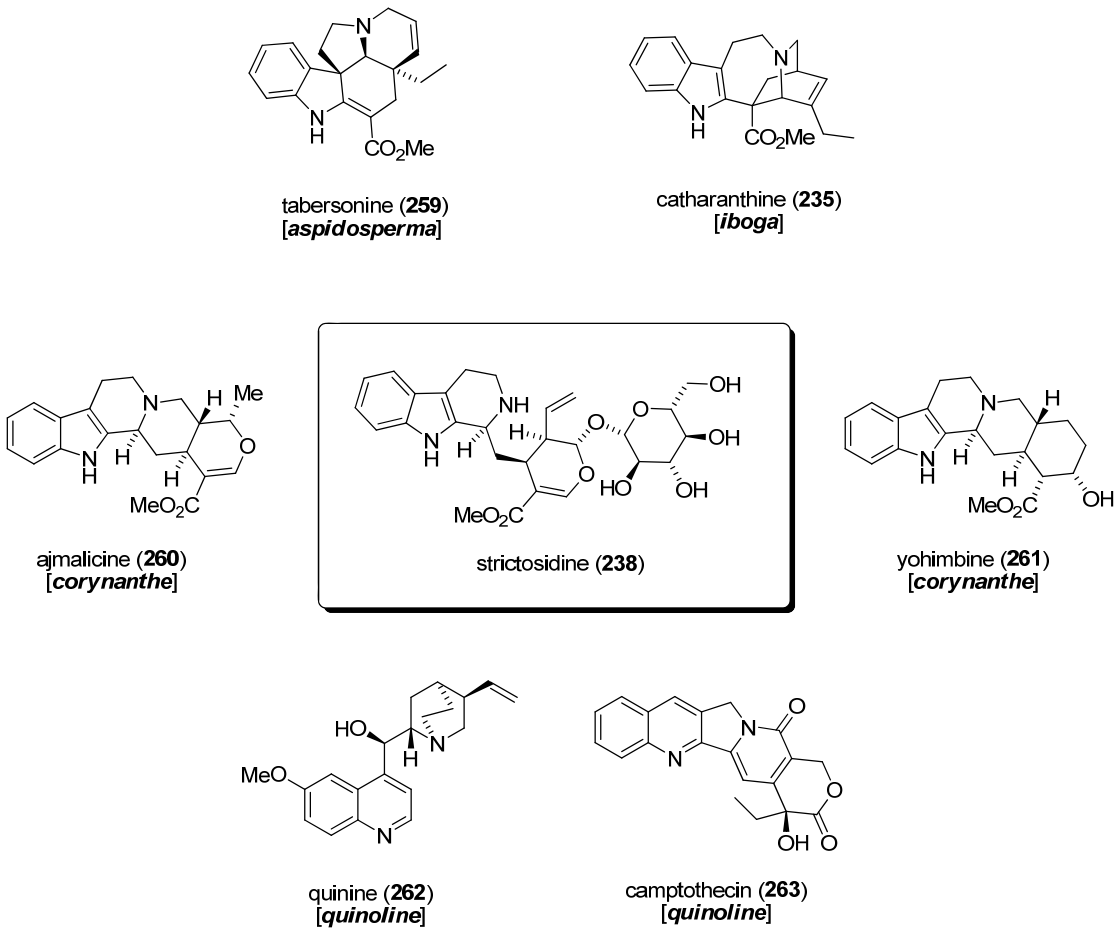
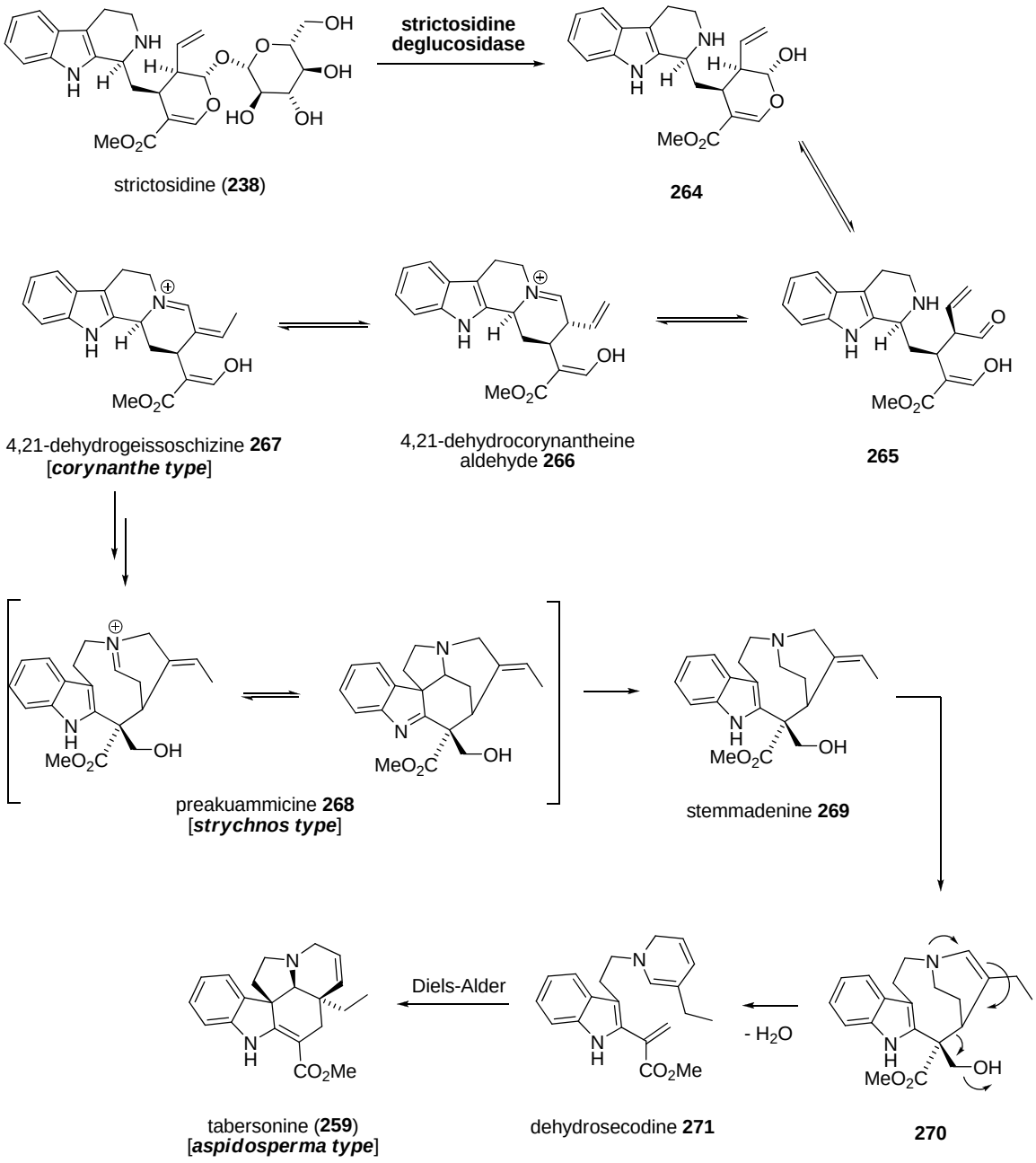


Figure 46. Major classes of terpene indole alkaloids derived from strictosidine.

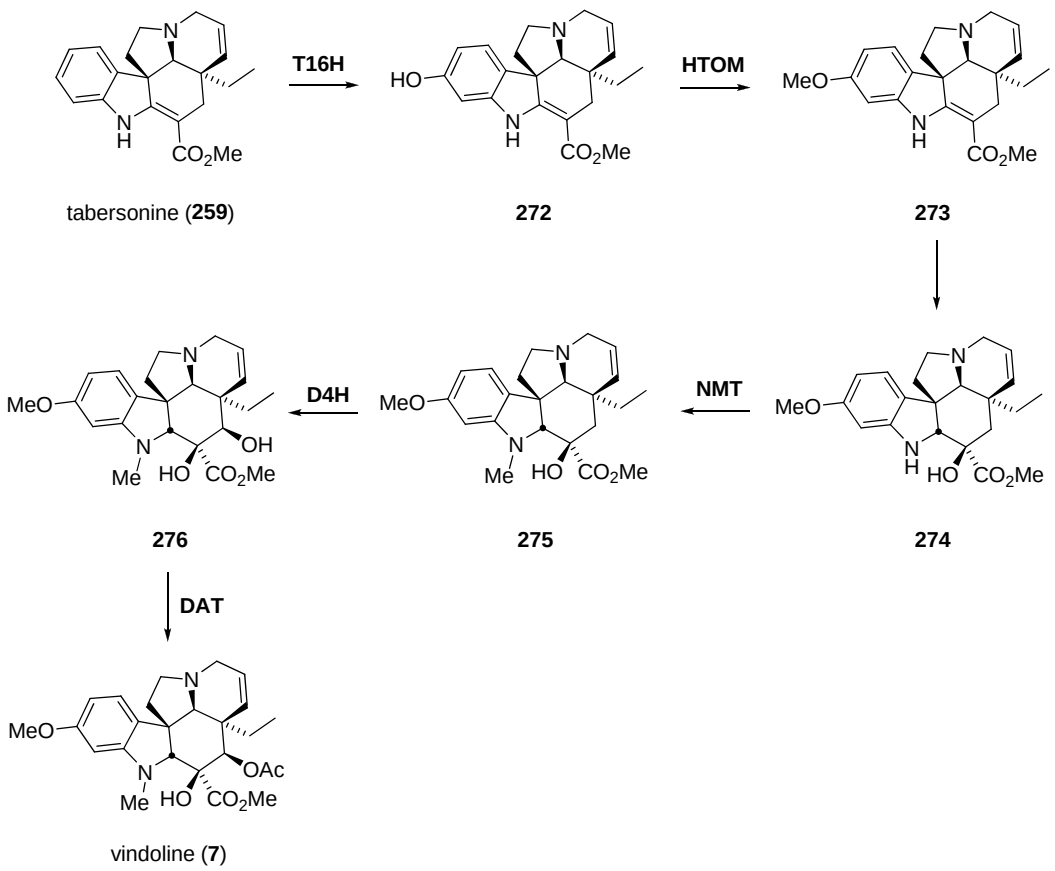
The deglycosylation of strictosidine (**238**) by strictosidine β-D-glucosidase reveals a reactive intermediate that serves as a point for biosynthetic divergence, Scheme 13.[187] Very little is known about the enzymatic catalysis after the deglycosylation of strictosidine (**238**) and leading up to tabersonine (**259**). The construction of the strychnos, corynanthe, iboga, and aspidosperma skeletons has been elucidated based on feeding studies of isotopically labeled substrates to seedlings and/or shoots and are thought to be derivatives of the corynanthe alkaloids.[188] These studies, conducted mainly in the 1960's and 1970's, have provided enough chemical structure information for a suggested biosynthetic pathway.[188-197] It is worth noting that Wenkert speculated that a general

biogenetic route to aspidosperma and iboga alkaloids occurred by a intramolecular Diels-Alder reaction of acrylic acid–enamine **271** years prior to any evidence that substantiated the claim.[193]



Scheme 13. Suggested biosynthetic pathway for strychnos, corynanthe, and aspidosperma type alkaloids.

Much more detail is known about the last six steps of the biosynthesis of vindoline (**7**) from tabersonine (**259**), Scheme 14. The hydroxylation of tabersonine (**259**) to 16-hydroxytabersonine **272** is performed by the cytochrome P450 monooxygenase tabersoninie-16-hydroxylase (T16H). The P450 enzyme T16H that is responsible for the oxidation of the indole nucleus has been cloned.[198, 199] Next the 16-hydroxy group is *O*-methylated to yield 16-methoxy-tabersonine **273** *via* 16-hydroxytabersonine-16-*O*-methyltransferase (HTOM), an enzyme that have been purified[200] and subsequently cloned.[201] The hydration of the acrylic double bond in **273** is to give 16-methoxy-2,3-dihydro-3-hydroxytabersonine **274** is currently an unknown enzymatic process, presumably some kind of hydratase. An *S*-adenosyl-L-methionine (SAM) – dependent *N*-methyltransferase (NMT) recently characterized and cloned is responsible for the methyl group transfer to the indole nitrogen to yield desacetoxyvindoline **275**.[202] The penultimate intermediate deacetylvindoline **276** is oxidized by the dioxygenase enzyme desacetoxyvindoline 4-hydroxylase (D4H) which has also been cloned.[203] The last biosynthetic step that yields vindoline (**227**) occurs by the acetylation of **276** by deacetylvindoline *O*-acetyltransferase (DAT).[204]



Abbreviations: T16H: tabersonine-16-hydroxylase; HTOM: 16-hydroxytabersonine 16-*O*-methyltransferase; NMT: *N*-methyltransferase; D4H: desacetoxyvindoline 4-hydroxylase; DAT: deacetylvindoline *O*-acetyltransferase.

Scheme 14. Vindoline biosynthesis from tabersonine.

The biochemistry involved in aspidosperma biosynthesis has been well established with several of the late-stage enzymes fully characterized and cloned, *e.g.* T16H, HTOM, NMT, D4H, DAT. Arguably, with regards to the biology of these processes, the most important discoveries have not been the biosynthetic pathways themselves but the localization of the pathways in the plants. These biosynthetic pathways do not exist within a single location in a plant cell or even within a single plant cell type. For example, aspidosperma alkaloids in *C. roseus* are produced in at least three cell types. The first few steps of the iridoid pathway take place in the internal phloem leaf

cells, phloem being the internally located tissue responsible for the transport of nutrients to all parts of the plant. The main set of biosynthetic transformations leading up to vindoline (**7**) occur in the leaf epidermis while the last two steps of the biosynthesis occurs in laticifer and idioblasts cells, cells typically found in leaves and bark where they have the function of storing non-living substances used for defense, excretion, and/or storage of reserves.[205]

Vinblastine (**13**) and vincristine (**14**) occur in extremely low levels in the periwinkle plant and enormous quantities of raw plant material are required for commercial production. Nearly 500 kg (dry weight) of plant material are needed for one gram of a mixture of active alkaloids. Vincristine (**13**) alone constitutes only 0.0003% by weight of dry plant material.[206] The structural complexity of the monoterpene indole alkaloids, in particular the bisindole alkaloids, and low natural abundance of these substances exemplify the need for the understanding of biosynthesis for eventual use in biotechnology. In terms of producing complex chemical entities on a commerically viable scale, the gap between chemistry and biology is being reduced by advances in microbiology and biotechnology. The future of synthetic biology, where recombinant microorganisms are designed to perform a series of molecular transformations, is gaining momentum with continued academic and industrial acceptance.

2.4.2 Biological Activity Profiles

Catharanthus roseus was initially investigated for its biological activity due, in part, to the reputation and folklore it has received from indigenous medicine in various parts of the world. In 1910 Peckholt described the use of *C. roseus* in Brazil as an

infusion of the leaves to control hemorrhage and scurvy, as a mouthwash to ease the pain of toothaches, and to clean and help heal chronic wounds.[207] In the Philippines the plant was reported as an effective oral hypoglycemic agent and in the former British West Indies as a treatment for diabetic ulcer. In South Africa and England the plant extracts were marketed under the names "Covinca" and "Vin-q-lin" as a treatment for diabetes.[208]

Two independent research groups began programs into the phytochemical properties of this plant, one at the Collip Laboratories at the University of Western Ontario and the other at Lilly Research Laboratories. The research group at Collip Labs. observed peripheral granulocytopenia and bone marrow depression in rats from certain alkaloidal fractions. This study eventually led to the isolation and identification of the sulfate of vinblastine (**13**), which was deemed the alkaloid responsible for the previous physiological observations as well as the causes for producing severe leukopenia, or lowering of white blood cell count, in rats.[209] Lilly researchers had been using mixtures that contained vinca alkaloids on mice with P-1534 leukemia. The studies showed a prolongation of life in mice that were subjected to the vinca alkaloid containing mixtures.[210] These initial studies led to the identification of additional alkaloids with potent biological activities of which vinblastine (**13**) and vincristine (**14**) will be discussed.

Although vindoline (**7**) exhibits weak inhibition of tubulin polymerization and high micromolar IC_{50} values for the inhibition of ATPase, a class of enzymes responsible for the degradation of adenosine triphosphate (ATP) to adenosine diphosphate (ADP), its bisindole alkaloid cousins overshadows its biological activity. Some of the biochemical effects observed from treatment of cells and tissues with bisindole alkaloids include the

disruption of microtubules, inhibition of protein synthesis and nucleic acids, alteration of lipid metabolism and the lipid content of membranes, the elevation of cyclic adenosine monophosphate (cAMP), and the inhibition of calcium-calmodulin-regulated cAMP phosphodiesterase. It is the antineoplastic activity, or anti-tumor activity, of the bisindole alkaloids vinblastine (**13**) and vincristine (**14**) that has garnered value as clinical chemotherapies. The anti–tumor activity of the vinca alkaloids is generally attributed to the disruption of microtubule formation that ultimately results in the degradation of mitotic spindles and metaphase arrest in dividing cells.[211, 212] The effects of the vinca alkaloids on the organization and function of microtubules has been heavily researched and it has been determined that each heterodimer of α,β-tubulin possesses a single high affinity and "vinca specific" binding site and several low affinity, nonspecific sites.[213] On the basis of *in vitro* microtubule assembly experiments it is assumed that the vinca alkaloids operate by more than one biological mechanism.[214, 215] At low concentrations vinblastine (**13**) inhibits the formation of microtubules by means of sub-stoichiometrically binding to high affinity sites at the ends of microtubules. At higher concentrations microtubule disassembly results from vinblastine (**13**) binding to heterodimers located along the surface of the microtubule. The binding to the low affinity sites along the microtubule surface occur through stoichiometric interaction with vinca–specific sites.

A well-described correlation between the cytotoxic actions of the vinca alkaloids and tumor cell resistance has been reported in the literature.[216, 217] The accumulation and retention of vinblastine (**13**) in cultured tumor cells was reported as the primary mechanism of drug resistance, not necessarily because of alterations in tubulin binding

sites.[218] The process of vinca alkaloid drug resistance is mediated by P-glycoprotein (Pgp). Similar to other known clinical anti–cancer drugs, Pgp binds vinca alkaloids and extrudes them from the tumor cell. As a result significant research has been formed at combination chemotherapy protocols in which Pgp modulators, or sacrificial substrates for Pgp, are used to increase plasma levels of the vinca alkaloid and increase their half-life.[219]

More specifically, vinblastine (**13**) has been approved as a component of combination therapy for the use in treatment of Hodgkin's lymphoma, non–Hodgkin's lymphomas, advanced mycosis fungoids, advanced testicular carcinoma, Kaposi's sarcoma and histiocytosis. Vincristine (**14**) has also been approved for the treatment of the same spectrum of Hodgkin's and non–Hodgkin's lymphomas, rhabdomyosarcoma, neuroblastoma, Wilms' tumor, Ewing sarcoma, melanoma, and small–cell lung cancer.

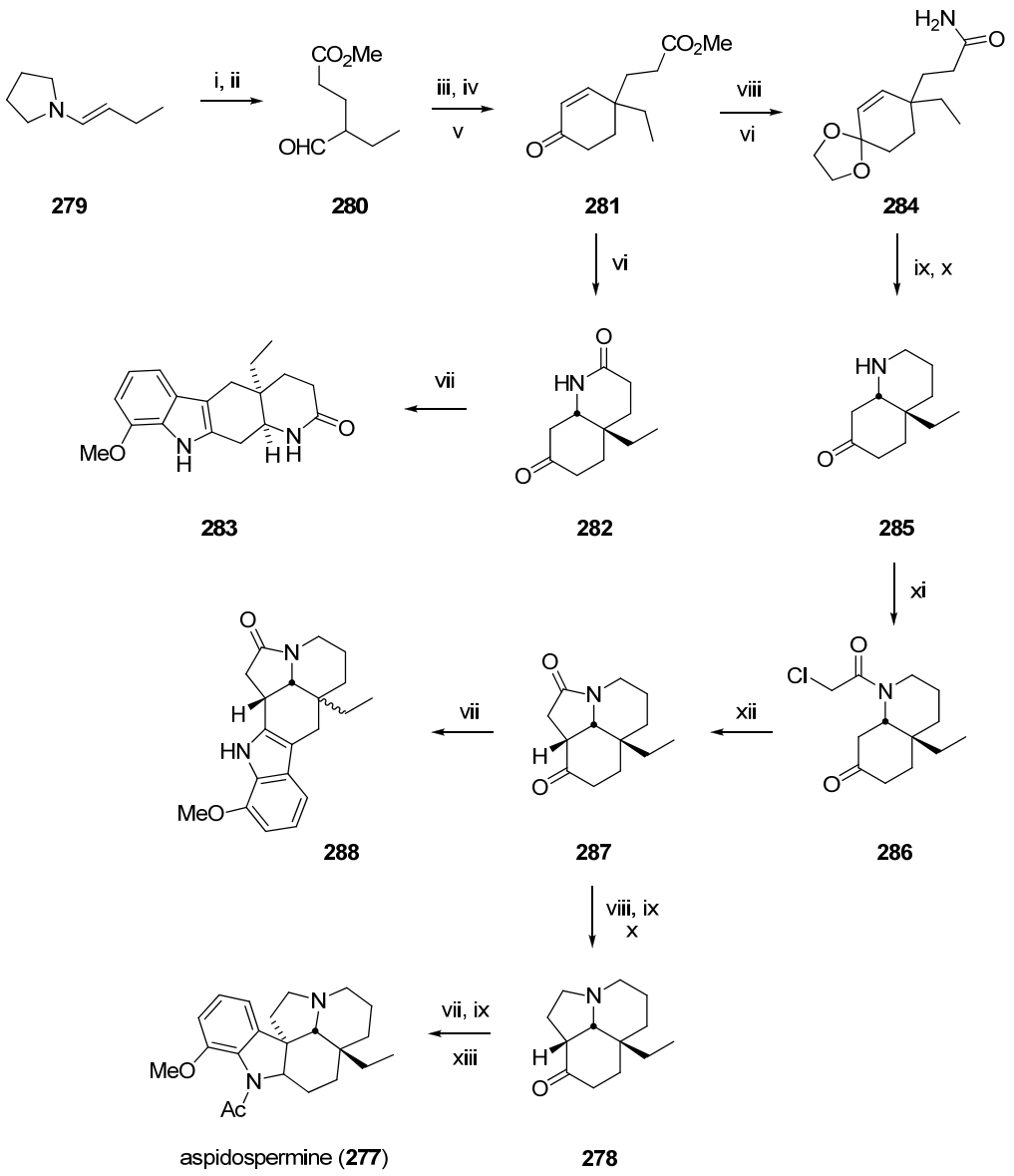
2.4.3 Selected Syntheses and General Strategies

Of the numerous strategies that have been used in the synthesis of aspidosperma alkaloids, the classic approach that Stork used in his synthesis of *rac*–aspidospermine (**277**)[6] became an approach used by several groups for the construction of the tricyclic terpenoid end of the molecule. Stork's strategy is the basis of the work presented in this document, *i.e*. the formation of a tricyclic intermediate similar to **278**, and, thus, the syntheses selected will also reflect the tricyclic intermediate–Fischer indolization approaches to aspidosperma alkaloids.

Stork (1963)

Stork's seminal work in the field of aspidosperma synthesis was reported in 1963 and stands as the first synthesis of aspidospermine (**277**), Scheme 15.[6] The initial part of the sequence utilizes Stork's enamine alkylation method[220] in which the enamine of butyraldehyde **279** was used in the Michael addition with methyl acrylate and after acid hydrolysis yielded aldehyde **280**. A second Michael addition of an enamine derived from **280** with methyl vinyl ketone followed by hydrolysis and aldol reaction provided enone **281**. Amidation with ammonium hydroxide and subsequent cyclization formed lactam **282** that was then subject to the classical Fischer indolization conditions yielding the undesired linear indole **283**. According to the report the outcome was not unexpected and the means to circumvent the linear indolization was to construct a tricyclic system by re-functionalizing ester **281**. The primary amide **284** was prepared and cyclized to give **285**. Acylation of **285** to **286** and substitution of the chloride with the enolate of **286** provided tricycle lactam **287**. In this case, Fischer indolization of tricycle **287** provided the kinetic ene–hydrazine and led to indole **288** instead of the desired thermodynamic outcome. Stork speculated that by reducing the amide functionality in **287** and, thus, increasing the number of tetrahedral centers, would allow for a more favorable transition state leading to the thermodynamic ene–hydrazine and the ultimate cyclization. His rationale proved correct when **278** was subjected to indolization to form the indolenine which was reduced and acetylated to yield *rac*-aspidospermine (**277**).

Several diastereomerics from Stork's work were later deduced by Ban *et al*. on the basis of chemical properties comparison during his synthesis of aspidospermine.[221]

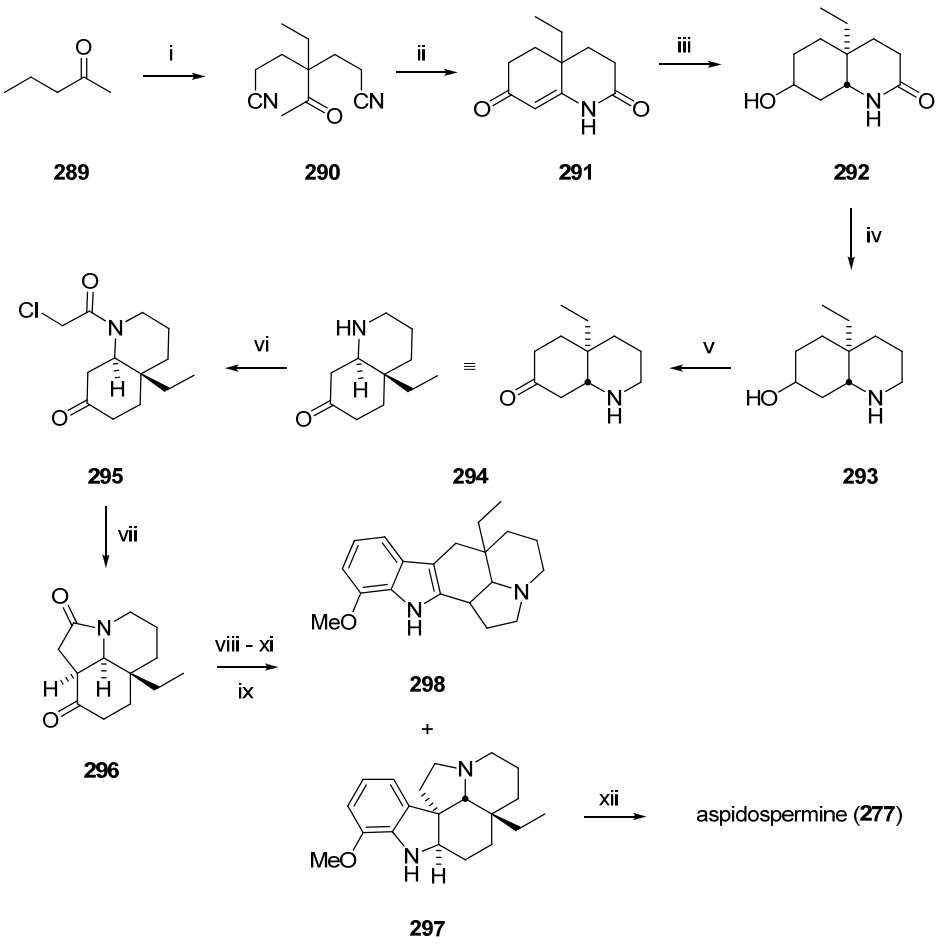


Reagents: (i) methyl acrylate; (ii) HOAc (aq), rt (67% from **279**); (iii) pyrroldine; (iv) methyl vinyl ketone; (v) HOAc (aq), Δ (48% from **280**); (vi) NH_4OH (aq), rt; (vii) *o*-methoxyphenylhydrazine, HOAC, Δ (30% from **282**); (viii) ethylene glycol, *p*TsOH; (ix) $LiAlH_4$; (x) H_3O^+; (xi) chloroacetyl chloride; (xii) KO*t*Bu, PhH; (xiii) Ac_2O.

Scheme 15. Stork's synthesis of *rac*-aspidospermine (1963).

Ban (1965)

The synthesis of aspidospermine (**277**) by Ban and co-workers shared a similar strategy to that of Stork's[6] but was significant in that a stereochemical divergence was identified by the comparison of synthetic intermediates.[221] Ban's synthesis began with the addition of pentanone **289** to two equivalents of acrylonitrile which, upon heating in concentrated sulfuric acid, cyclized to vinylogous amide **291**, Scheme 16. The reduction of **291** with palladium/H_2 in a basic medium afforded alcohol **292** of which the amide moiety was deoxygenated with $LiAlH_4$ to **293**. The oxidation of **293** to **294** using Oppenauer's conditions[222] intercepted an intermediate utilized in Stork's synthesis, namely **285**, and provided an opportunity to compare physical properties, Figure 47. Despite the evident difference in physical characteristics between Stork and Ban's intermediates the synthesis was continued in the hope to validate the synthetic pathway from presumed (at the time) diastereomers. From the point of ketone **294** the synthesis mimics that of Stork's by the formation of tricyclic lactam **296** and subsequent chemistries that lead to the Fischer indolization product deacetyl aspidospermine **297**. Acylation of **297** yielded aspidospermine (**277**) whose spectral data matched those of the natural alkaloid.



Reagents: (i) acrylonitrile (2 equiv); (ii) 80% H_2SO_4, 150 - 160 °C (80%); (iii) Pd, alkaline medium, H_2 (g) (79%); (iv) $LiAlH_4$, THF, 75 °C (81% for **293**); (v) KO*t*Bu, cyclohexanone (74%); (vi) chloroacetyl chloride; (vii) K*t*OBu; (viii) ethylene glycol, H^+; (ix) $LiAlH_4$; (x) H_3O^+; (xi) *o*-methoxyphenylhydrazine, HOAc, 90 - 95 °C; (xii) Ac_2O.

Scheme 16. Ban's aspidospermine synthesis (1965).

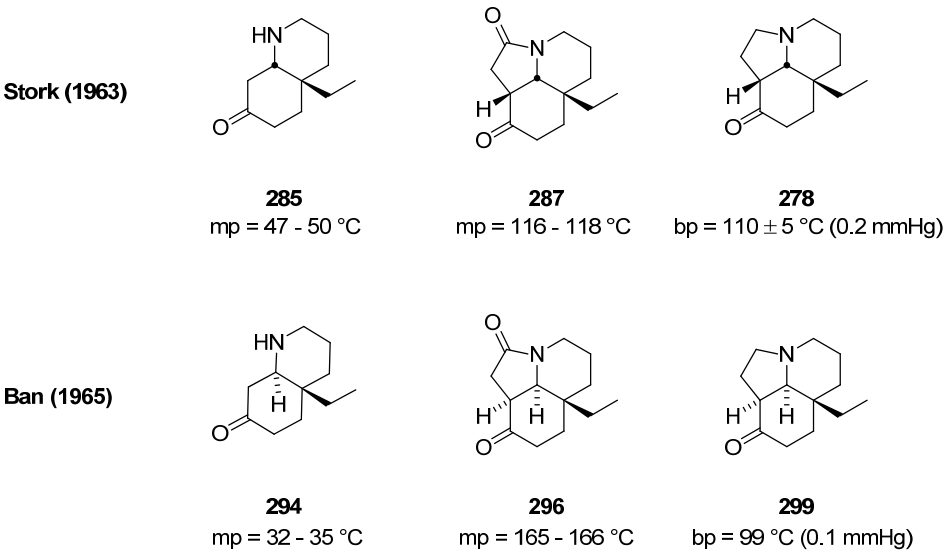


Figure 47. Comparison of physical properties.

Regardless of which diastereomer is prepared, Stork's **278** or Ban's **299**, the cyclization reaction that yields indolenine **300** and **301** is immediately followed by a retro-Mannich process[6] that defines the stereochemistry of that ring system. This redundant process was originally proposed by Smith and Wróbel.[223] The imminium **302** ultimately equilibrates to the more stable pentacyclic core of aspidospermine (**277**) and substantiates Stork's claim regarding the ambiguity of the chiral centers involved in the indolization process, namely during the formation of the ene-hydrazine, Figure 48. Stork's statement regards the sole dependency of the quaternary center's (C-22, aspidospermine numbering) stereochemistry on the outcome of the final product of the indolization. The retro-Mannich equilibration is favored at elevated temperatures and has been used in the syntheses of other aspidospermine and quebrachamine-like natural products.

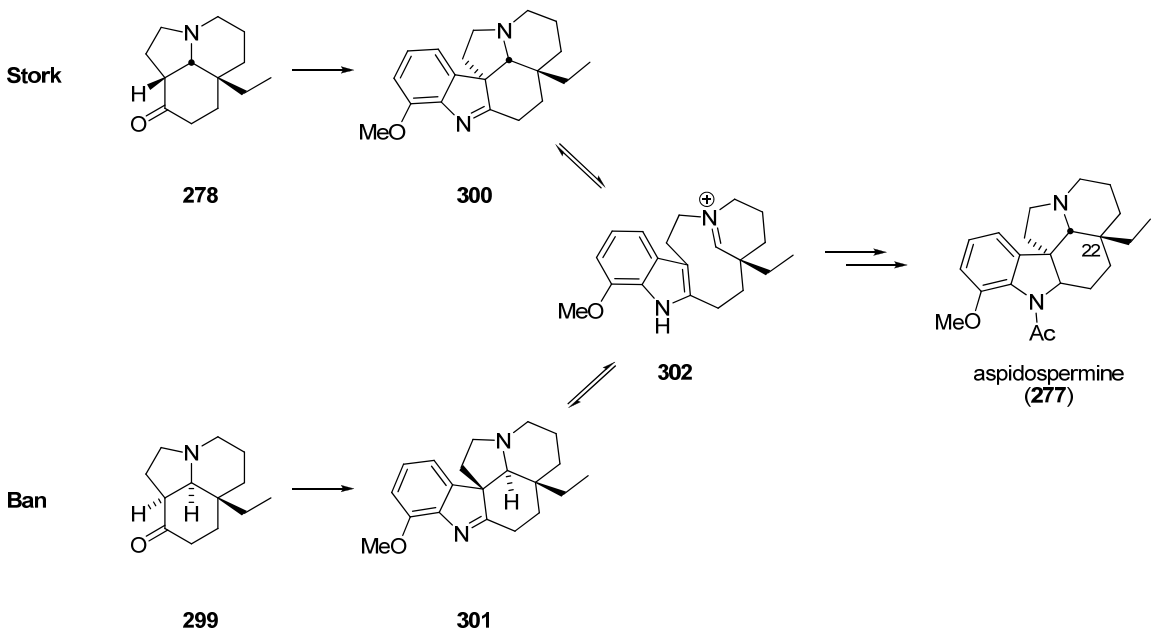


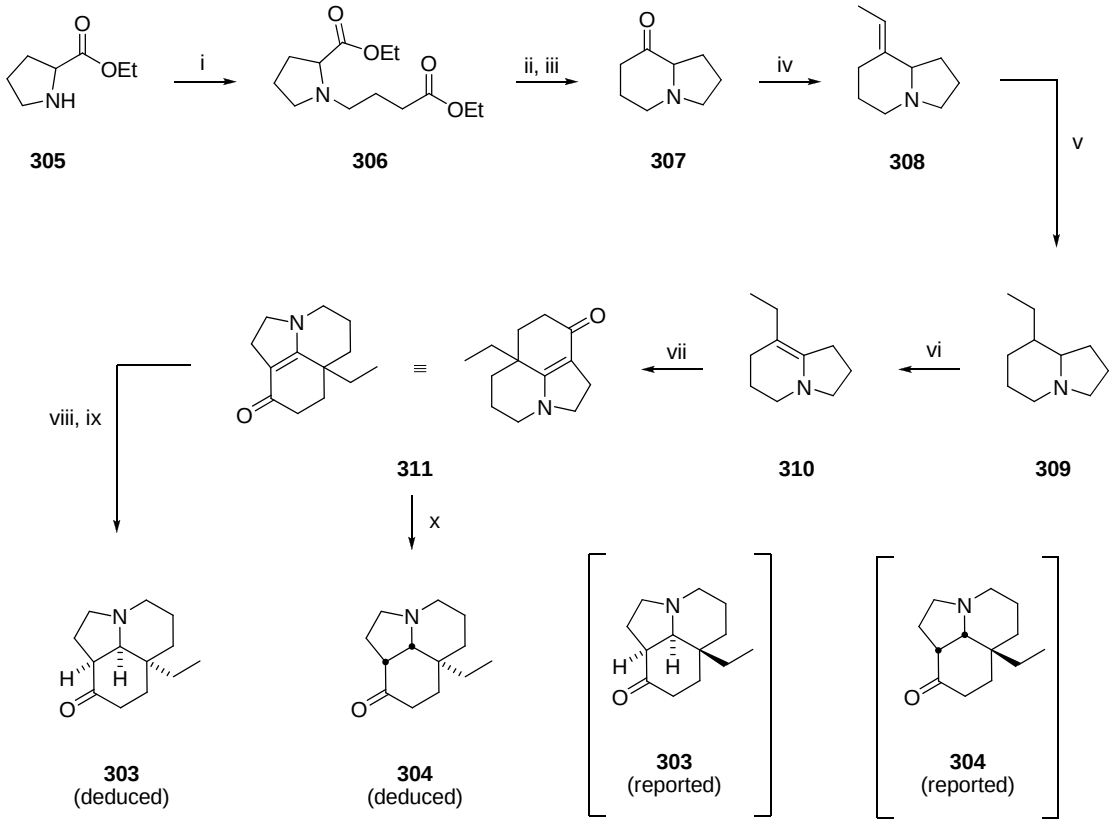
Figure 48. Retro-Mannich equilibration of diastereomers to aspidospermine (**277**).

Kuehne (1966)

Kuehne and Bayha proposed the synthesis of two additional diastereomers of the "Stork" tricyclic ketone in 1966.[224] Although comprehensive investigation could not confirm the identity of the structural isomers **303** and **304**, Scheme 17, spectroscopic comparison with **278** and **299** showed similarities and Kuehne claimed that the diastereomers could be paired based on *trans* or *cis*-fused six membered rings: *trans*-fused **278**/**303** and *cis*-fused **299**/**304**. The reaction scheme is shown with stereochemistry deduced from other isomers prepared (and validated). There is uncertainty because of the lack of physical characteristics and conflicting assignments with previous reports by Stork and Ban.

Kuehne and Bayha's synthesis started with the alkylation of ethyl ester of proline **305** with γ-bromo ethylbutyrate to give **306**. Compound **306** underwent a Dieckmann

condensation followed by decarboxylation to yield bicycle **307**. Annulated product **307** by was subjected to Wittig olefination and reduced to yield **309**. The tricyclic ketone **311** was constructed *via* enamine condensation of **310** with methyl acrylate. This was the stepping off point for the preparation of diastereomers **303**/**304**. Vinylogous amide **311** was reduced over a platinum catalyst and re-oxidized to produce **303** while, alternatively, **311** was treated with $LiAlH_4$ to yield **304**.

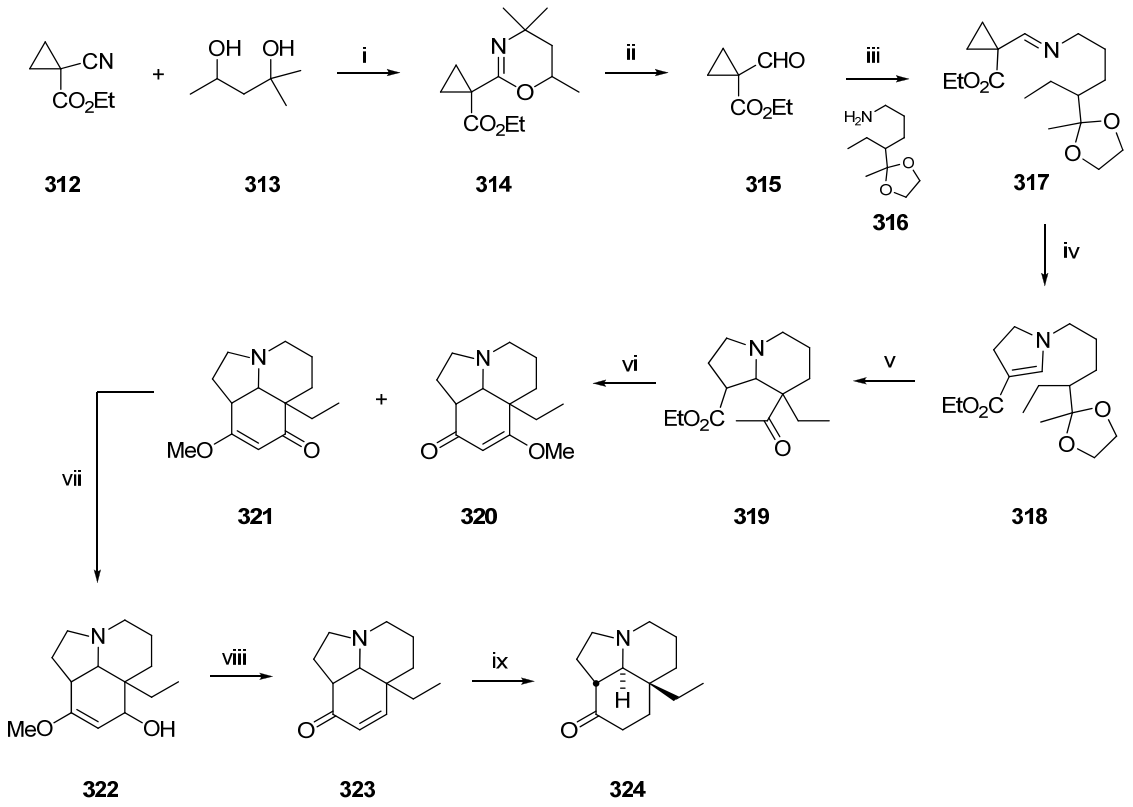


Reagents: (i) γ-bromo ethylbutyrate (80%); (ii) NaH; (iii) H^+, hydrolysis, decarboxylation (60% from **306**); (iv) triphenylphosphoranylidene ethane (40%); (v) Pd/C, H_2 (69%); (vi) $Hg(OAc)_2$ (60%); (vii) methyl acrylate (41%); (viii) Pt/H_2, EtOH, HOAc; (ix) $Al(iOPr)_3$; (x) $LiAlH_4$.

Scheme 17. Kuehne's synthesis of tricyclic ketone **303**/**304** (1966).

Stevens (1971)

R. V. Stevens's formal synthesis of (±)-aspidospermine (**277**) provided yet another strategy to construct the tricyclic ketone intermediate utilized by Stork, Ban, and Kuehne.[225] Stevens's synthesis is highlighted by the thermal rearrangement of cyclopropyl imine **317** prepared from the condensation of cyano ester **312** with 1,3-diol **313** followed by subsequent chemistry, Scheme 18. The formation of the pyrroline **318** allowed for the formation of the annulation product **319** which, upon treatment with sodium methoxide/methanol, was transformed to a pair of separable tricyclic enol ethers **320**/**321** (28:72). The major component **321** was reduced to **322** and hydrolyzed – dehydrated to enone **323**. The formal synthesis of *rac*-aspidospermine (**277**) was completed upon hydrogenation over palladium and matching spectral data to authentic samples.



312 313 314 315 316 317 318 319 320 321 322 323 324

Reagents: (i) H_2SO_4 (64%); (ii) $NaBH_4$; then H_3O^+ (61%); (iii) **316**, PhH (86%); (iv) NH_4Cl, 160 °C (82%); (v) HCl/Et_2O; then aq. K_2CO_3 (86%); (vi) NaOMe, MeOH; then anhyd. HCl (93%, 28:72 **320/321**); (vii) $LiAlH_4$ (96%); (viii) H_3O^+ (70%); (ix) Pd/C, H_2.

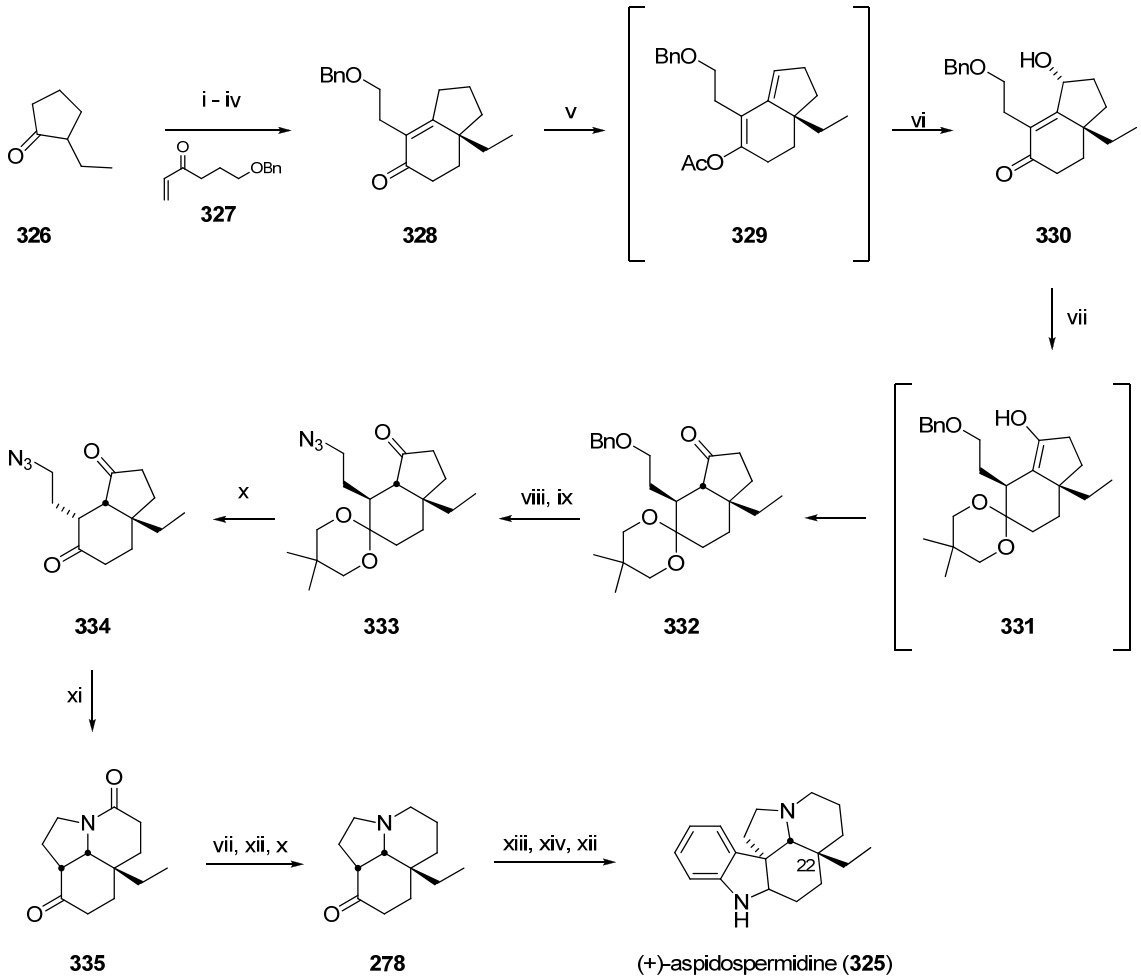
Scheme 18. Stevens' formal synthesis of aspidospermine (1971).

Aube (2000 / 2005)

Although Aubé and co-workers's efforts toward the synthesis of (+)–aspidospermidine (**325**) were arduous in planning, lengthy (22 linear steps), and suffered from low overall yield (1.1 %) the technical display of chemistries used to construct the tricyclic ketone is noteworthy and represents a modern approach to the Stork's classical intermediate **278**.[226, 227] The synthesis began with an asymmetric version of the Robinson annulation developed by d'Angelo.[228] The enamine that resulted from the condensation of (*S*)-methylbenzylamine and 2-ethyl cyclopentanone **326** was used to alkylate Michael

acceptor **327**, Scheme 19. Acid hydrolysis followed by cyclization with sodium methoxide/MeOH yielded bicyclic enone **328** in good enantiomeric excess (84 – 86%), to which was increased to enantiopurity downstream. The series of transformations that led to **328** established the only stereocenter (C-22, aspidospermine numbering) critical for the preparation of the natural product, Figure 48. The gamma position of the enone in **328** required a higher oxidation state in order to establish a basis for the future Schmidt reaction. To this end **328** was treated with isopropenyl acetate to generate the dienol acetate **329** and subsequently oxidized with potassium peroxymonosulfate (Oxone) to yield γ-hydroxy enone **330**. It was determined through other failed routes (not shown) that although the enone in **330** needed to be fully saturated in the α,β-position, the nucleophilicity that resulted from the enolate presented problems during attempts to install the azide moiety, *e.g*. competitive cyclopropanation. Instead, Aubé and co-workers performed a "redox ketalization" using bis(trimethylsilyl)neopentyl glycol and trimethylsilyl triflate (TMSOTf) to form ketone **332 (**12:1 mixture of diastereomers) through enol **331**, while at the same time protecting the side chain from epimerization. Functional group interconversion of the benzyl group in **332** to azide **333** was completed *via* hydrogenolysis and a subsequent Mitsunobu reaction with hydrazoic acid. The latent ketone in acetal **333** was liberated with lithium tetrafluoroborate ($LiBF_4$) to yield the Schmidt intermediate **334** as a 10:1 mixture of diastereomers (major shown). The Schmidt reaction of azido diketone **334** assisted by titanium tetrachloride ($TiCl_4$) proved to be regioselective and provided a single diastereomer of **335** (84% ee). A series of functional group manipulations led to the eventual synthesis of Stork's tricyclic ketone **278** and completed the formal synthesis of aspidospermine (**277**). To extrapolate from

tricycle **278** to (+)-aspidospermidine (**325**) Fischer indolization was performed at elevated temperatures with phenylhydrazine in acetic acid. The resulting indolenine was reduced to form (+)-aspidospermidine (**325**).



Reagents: (i) (*S*)-α-methylbenzylamine; (ii) **327**, hydroquinone, $ZnCl_2$, Et_2O, reflux; (iii) aq. HOAc (10%); (iv) NaOMe, MeOH, reflux (49% from **326**, 84 - 86% ee); (v) isopropenyl acetate; (vi) Oxone, acetone (74% from **328**); (vii) bis(trimethylsilyl)neopentyl glycol, TMSOTf, DCM, 0 °C → rt (69%); (viii) Pd/C, H_2 (93%); (ix) HN_3, PPh_3, DEAD, PhH, 0 °C → rt (82%); (x) $LiBF_4$ (89%) (for **278**: 67% from **335**); (xi) $TiCl_4$, DCM (82%); (xii) $LiAlH_4$, THF, reflux (for **325**: 51% from **278**); (xiii) phenylhydrazine; (xiv) HOAc, reflux.

Scheme 19. Aubé's formal synthesis of aspidospermidine (2005).

The classical synthetic routes described in this section are linear in the means at which the target alkaloids are constructed. The bond-by-bond construction strategy is still

commonly used in modern synthesis and those tactics will continue to be implemented when necessary. However, more efficient tandem processes are generally used in the strategic planning of the synthesis of complex natural products. Some examples of these practices will be outlined by the work of Fowler, Zard, and Marino in the coming chapters.

3 Results and Discussion

3.1 Introduction

The following chapter summarizes the efforts made by this author to contribute examples of chemoenzymatic syntheses and showcases the versatility of (–)-3,5-cyclohexadiene-1,2-diol-1-carboxylic acid **4**, generated enzymatically from benzoic acid by the mutant organism *Ralstonia eutropha* B9. The chapter is divided into four main bodies of work and that include: 1) enzymatic dihydroxylation of benzoic acid by *R. Eutropha* B9, 2) the synthesis of (–)-idesolide, 3) the synthesis of an iminosugar, and 4) approaches toward the synthesis of Vinca alkaloids.

3.2 Dihydroxylation of Benzoic Acid by *Ralstonia eutropha* B9

The use of the whole-cell fermentation technology to generate enantiomerically pure molecules and the utility of these compounds in enantioselective syntheses has proven to be a powerful and versatile combination.[19, 74, 77] As a part of the sustained effort to demonstrate the benefits of a combined chemoenzymatic approach to organic synthesis, substrates for enzymatic oxidation are sought that would result in useful chiral molecules. To this end the mutant organism *R. eutropha* B9, discovered in 1971 by Reiner[42, 229] and exploited by Myers in 2001,[66] was utilized as the vehicle for benzoate dioxygenase (BZDO) for the accumulation of (–)-3,5-cyclohexadiene-1,2-diol-1-carboxylic acid **4** in fermentations with benzoic acid. The research groups of Mihovilovic, Lewis, and Widdowson have also utilized the *R. eutropha* B9 cell lines toward chemoenzymatic synthesis.[67-69, 72, 73, 77, 230]

The production of *ipso* diene diol **4** is fairly robust and specialized equipment is not required. Like all biotransformation processes that utilize aromatic compounds as substrates, the technique relies on a fed-batch feeding regime to prevent substrate inhibition. In doing so high cell densities can be achieved and metabolic efficiencies can be maximized. In 2001 Myers *et al*. depicted a rudimentary process consisting of a 55 gallon drum (80 L working volume) that was capable of generating greater than 200 grams of *ipso* diol **4**.

In 2010 Mihovilovic and co-workers presented a significant improvement of the biooxidation process by establishing the correlation of oxygen consumption with substrate metabolism, a well-known phenomenon that occurs during enzymatic dihydroxylation.[69] Fine tuning of the substrate and carbon source feed limits were established for the fed-batch process that consisted of small additions of both substrate and carbon source into the ferment broth over several days. The implementation of D-fructose as a carbon source in place of succinate was also established and, in conjunction with a controlled substrate introduction, allowed for metabolite titers of up to 14.4 g/L on 2 L scale. The single most valuable and practical information from the report was on the isolation of the labile *ipso* diol **4**. Traditionally, and with most other metabolites derived from enzymatic dihydroxylation, *cis*-dihydro diene diols are isolated from basic ferment broth by exhaustive liquid-liquid extractions with an organic solvent. The tendency for *cis* diene diol metabolites to aromatize *via* dehydration is high unless careful measures are taken to limit exposure to acidic environments, either on macro- or micro-scale, even as far as to base-washed ethyl acetate (EtOAc) used in the extraction process. The dehydration of the *cis*-diene diols is self-propagating (autocatalytic) as a result of

accumulation of phenol of type **336**, Figure 49. To circumvent the two re-aromatization pathways leading to the degradation of *ipso* diene diol **4** to phenol **338** and salicyclic acid (**339**), Figure 49, Mihovilovic concentrated the neutral aqueous ferment broth to less than 1 L and precipitated the carboxylate of **4** with the addition of isopropanol (*i*PrOH). This method yielded a mixture of inorganic salts and the mixed potassium/sodium carboxylate **337**. The carboxylate salt **337** was found to be bench stable for more than six months without any observable degradation. This particular method of isolation proved to be irreproducible on larger scales (*e.g.* 8.5 L working volume) and it became more convenient to concentrate the ferment broth to dryness after centrifugation of cell matter. The composition of the mixture that contained diol carboxylate **337** was established quantitatively using the internal standard method by NMR and yields in the range of 18 – 22 g/L were consistently observed.

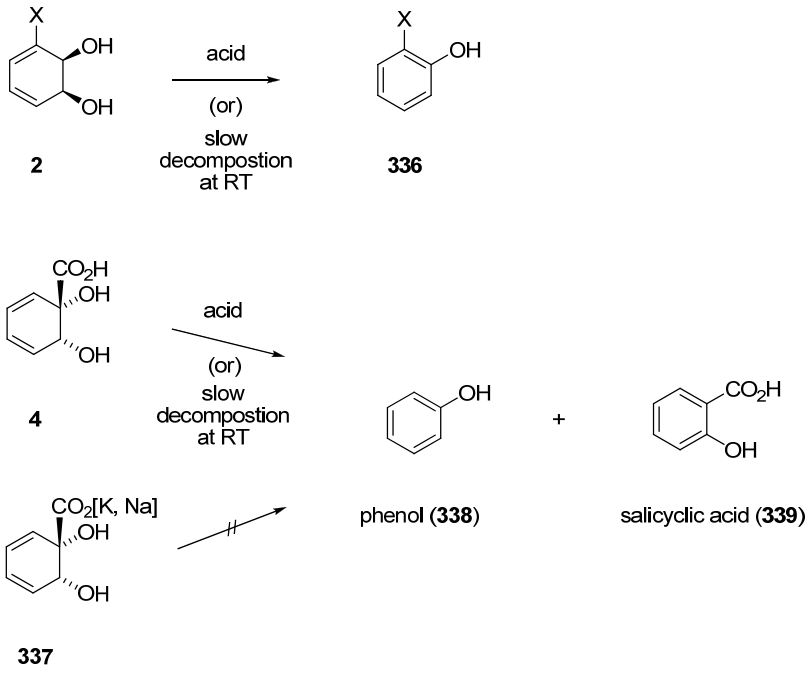


Figure 49. Chemical degradation of *cis* diene diols.

If the protonated carboxylic acid was needed as an intermediate, an aqueous solution of **337** could be prepared, acidified at 0 ˚C, and quickly extracted with ethyl acetate without too much danger of re-aromatization.

3.3 Chemoenzymatic Synthesis of (–)-Idesolide from Benzoic Acid

In 2010 back to back reports by Iwbuchi[82] and Kuwahara[83] were released on the synthesis of idesolide (**5**). The skeletal features, substitution pattern, and degree of oxygenation contained in idesolide (**5**) show an obvious resemblance to the *ipso* diene diol **4**. Two possible routes were envisaged for the synthesis of the monomeric precursor **8**, a previously established synthetic intermediate for idesolide, and that would allow for an efficient synthesis. The most concise route consisted of a direct isomerization of diene diol ester **340** to α-hydroxy ketone **8**, an overall neutral redox event, Figure 50. A second plan involved the selective reduction of the olefin distal to the ester group in diene ester **340** followed by a subsequent oxidation step.

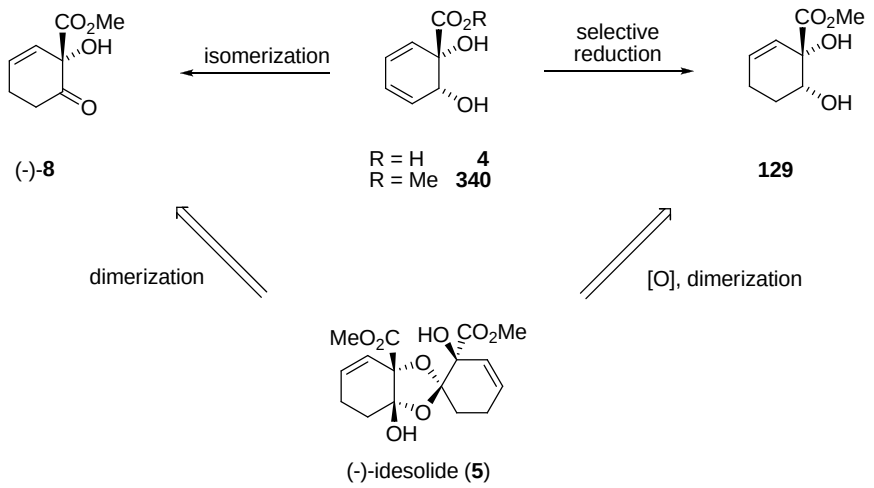
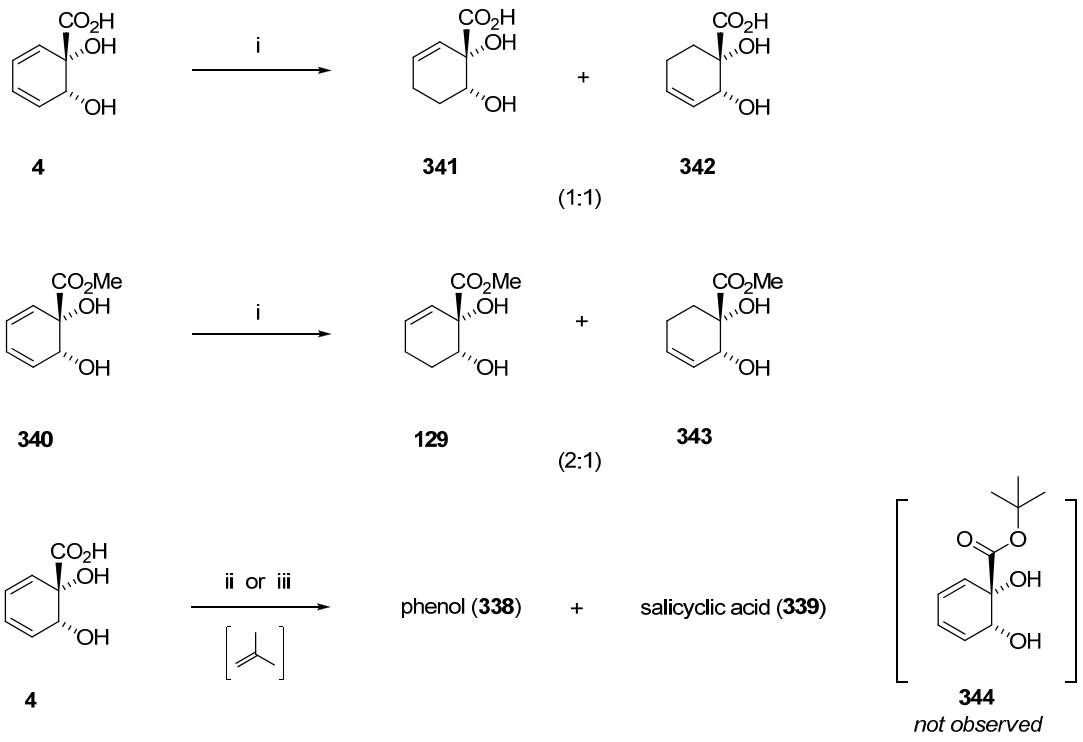


Figure 50. Idesolide retrosynthesis.

As a start to the synthesis of (-)-idesolide (**5**), carboxylic acid **4** and methyl ester **340**, derived by the treatment of **4** with diazomethane, were probed for selective reduction of the distal alkene with diimide. The Hudlicky group has used diimide, derived from the decarboxylation of potassium azodicarboxylate (PAD),[231] to reduce olefins in other syntheses that used diene diol metabolites obtained from the fermentation of arenes.[232] Diimide shows little electronic preference toward reactions with *cis* alkenes, as long as the alkene is not highly polarizable, and selectively is typically a result of the steric environment of the substrate. In the case of carboxylic acid diol **4** and methyl ester diol **340** the additional of steric bulk provided a slight increase in selectivity for the methyl ester **340**. A 1:1 ratio of reduced products **341** and **342** were observed when carboxylic acid **4** was treated with diimide while a 2:1 ratio in favor of the desired distally reduced olefin **129** was observed, Scheme 20. Separation of the two alkene isomers was possible on silica gel and the result provided some precedence for the synthetic route. To further demonstration the influence of steric bulk on the diimide reduction the preparation of the *tert*-butyl ester of **4** was attempted. Attempts at the derivatization of the carboxylic acid **4** as the *t*-butyl ester **344** were not successful and the failure was attributed to the incompatibility of the known methodology used to generate isobutylene *in situ*, *e.g.* dehydration of *tert*-butanol (*t*BuOH) with catalytic or stoichiometric sulfuric acid (H_2SO_4).[233, 234]

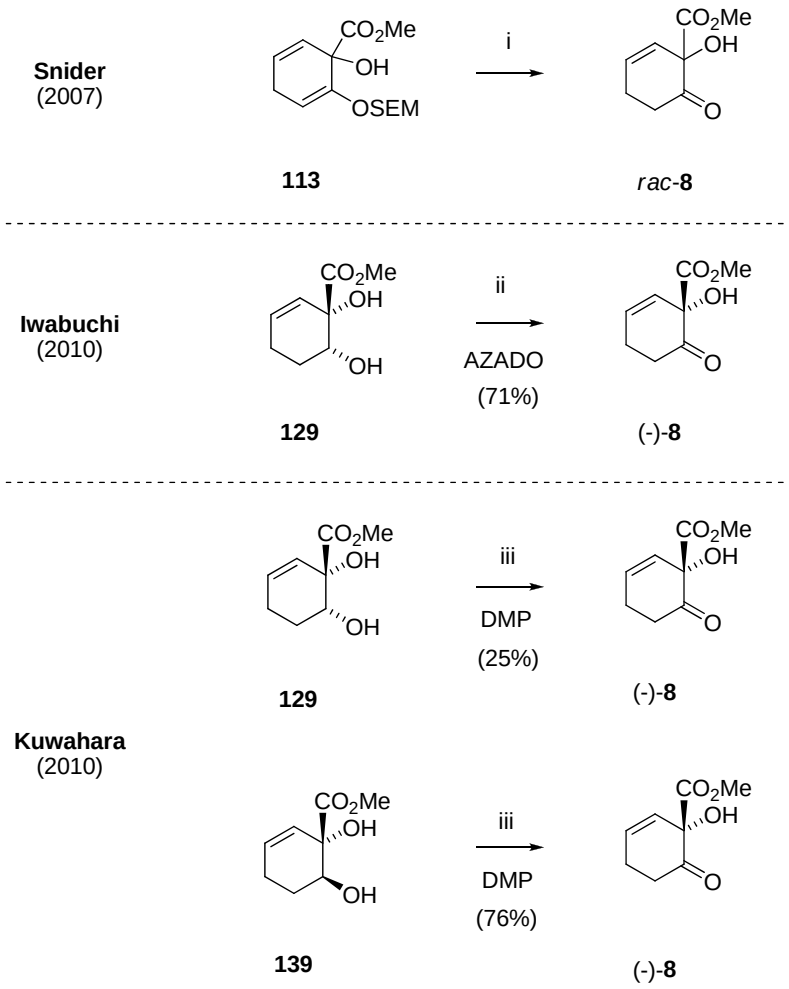


Reagents: (i) potassium azodicarboxylate, HOAc (glac), MeOH, 5 °C → rt (for **129**: 64%; for **343**: 25%; 89% overall yield for **129**/**343**); (ii) $MgSO_4$, H_2SO_4, *t*BuOH, DCM; (iii) *t*butyl acetoacetate, H_2SO_4 (cat).

Scheme 20. Reduction of distal olefin in ester **340**.

With *cis*-diol **129** readily available the oxidation to yield monomer **8** was screened. Snider, Iwabuchi, and Kuwahara approached the common monomeric intermediate **8** in slightly different ways in order to combat the problematic and sometimes labile nature of *cis*-1,2-diols toward oxidation. Snider utilized a completely different strategy in forming **8**. By means of dearomatization of SEM-protected methyl salicylate **112** *via* Birch reduction, Snider and co-works were able to generate SEM-enol ether **113**.[81] In doing so, **113** was unmasked as the α-hydroxy ketone **8** by a Lewis acid mediated desilylation – isomerization reaction, Scheme 21. Iwabuchi's choice of oxidant came about serendipitously prior to the improvement of the reaction conditions with *N*-

oxyl AZADO **115**, Figure 31. *N*-Oxyl based-oxidants impart a mild oxidative environment and the bulky and rigid nature of the adamantane-like skeleton of **115** was able to provide advantage over any participation from the vicinal alcohol.[82] Alternatively, Kuwahara and co-workers prepared a set of chromatographically separable epoxy alcohols **137α**/**137β** (1:5) that were used to prepare *trans*-1,2-diol **139** (from **137β**), Scheme 4.[83] t*rans*-Diol **139** underwent oxidation with Dess-Martin periodinane (buffered) in high yield (76% over two steps) without any interference from the neighboring *trans* alcohol . Comparatively, Kuwahra extrapolated the minor epoxy alcohol **137α** to *cis*-diol **129** and subjected it to the same oxidative conditions as **139** for a significantly reduced product yield (25%).



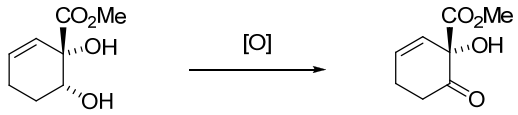
Reagents: (i) $MgBr_2{\cdot}Et_2O$, Et_2O, $MeNO_2$, rt (71%); (ii) AZADO **115**, PS-BAIB, CH_2Cl_2 (71%); (iii) DMP, $NaHCO_3$, CH_2Cl_2 (76% from **139**; 25% from **129**).

Scheme 21. Comparison of yields for monomer **8**.

Glycol cleavage is a well-known and often desired transformation in organic chemistry. Taking into consideration the required stereochemical relationship for 1,2-diol cleavage to occur, the comparative yields obtained by Kuwahara and co-workers for the oxidation of **129** versus **139**, the somewhat exotic choice of the oxidant by Iwabuchi, and finally, the lack of other common oxidants that were not attempted and/or reported led to the speculation that the *cis*-diol moiety in **129** was prone to cleavage and over oxidation. Iwabuchi's oxidation protocol using AZADO **115** in catalytic amount and polymer bound

bis(acetoxy)iodobenzene (BAIB) as the bulk oxidant proved unreliable and low yields of an equally low quality of **8** (85% pure by NMR analysis) were ever obtained. However, the reaction conditions did serve as a means to access a standard sample of **8** that could be used for further screening purposes. With a standard sample of **8** in hand a reagent screen was performed for the oxidation of *cis*-diol **129** to monomer **8** and is outlined below, Table 8.

Table 8. Reagent screen – oxidation of **129**.



129 (-)-**8**

Entry	**Oxidant**	**Result** (% yield)
1	Dess-Martin periodinane	**129** (30%) **8** (5-10%) cleavage/over oxidation
2	Collin's reagent	15% recovered **129** cleavage/over oxidation
3	AZADO (cat.), PS-BAIB[82]	**8** (30%)
4	Corey-Kim procedure[235]	complex mixture
5	oxoammonium TEMPO (1 equiv)[236]	**129** **8** (trace)

		complex mixture
6	Oppenauer oxidation ($AlMe_3$, nitrobenzaldehyde, $PhMe/CH_2Cl_2$)[237]	no reaction
7	CrO_3/Ac_2O, CH_2Cl_2 (-40 ˚C, -20 ˚C, 0 ˚C)	**8** formed initially. Decomposition during workup, *e.g*. concentration, neutralization yields complex mixture and additional attempts to trap as the hydrazone failed.
8	IBX, EtOAc, 80 °C (heterogeneous mixture)	**8** (27%) IBX-3° alcohol adduct
9	Fetizon's reagent (Ag_2CO_3/Celite)[238]	cleavage, rearomatized products
10	Dimethyldioxirane (DMDO)	epoxidation
11	tetrapropylammonium perruthenate (TPAP)[239]	**8** (trace)
12	$BaMnO_4$	no reaction
13	2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ)	no reaction
14	Swern oxidation[240]	**129** **8** (12%)
15	Mukaiyama oxidation[241]	no reaction
16	IBX, DMSO, rt	**8** (40 – 60%)

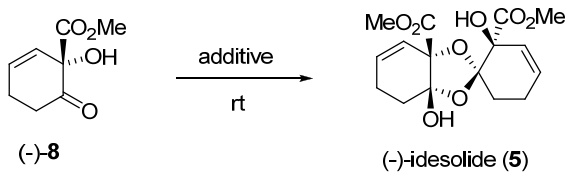
The oxidation of **129** was not trivial although several sets of conditions were successful in generating the α-hydroxy ketone **8**. *o*-Iodoxybenzoic acid (IBX) was chosen as the oxidant for the transformation based on the reaction profile presented during the screening process and the known inhibition of the 1,2-diol cleavage pathway.[242] Heterogeneous conditions (IBX in EtOAc, 80 ˚C) provided the most convenient workup of simply filtering off residual IBX and the insoluble reduced by-product iodosobenzoic acid (**345**) (IBA). However, there was always an inseparable impurity in appreciable amounts that could not be purged. The assumption, based on NMR analysis and ultraviolet (UV) activity of crude mixtures, was that the impurity was some form of IBX adduct with the tertiary alcohol in **129/8**. To this end, homogenous reaction conditions (IBX in DMSO, rt) were used for the oxidation and the reaction proceeded smoothly at ambient temperature. Dimethylsulfoxide (DMSO) presented other known challenges for workup, namely the high dielectric constant and high boiling point. The isolation of **8** from IBA by chromatographic methods was not possible, nor was liquid-liquid partitioning with diethyl ether successful. Yields were in the range of 20 – 30% using this workup. In order to circumvent further diminishment of yield through repetitive purifications, the IBA was precipitated by the slow addition of the reaction mixture into a stirred volume of water (2.25 x volume of DMSO). The inverse addition was necessary to avoid gum formation and entrapment of **8**. This improved workup procedure provided consistent yields in the 55-60% range with an improved quality of product **8**.

The final step of the synthesis of (-)–idesolide (**5**) was the dimerization of monomer **8** and this was performed using Kuwahara's reported conditions of dispersed sodium bicarbonate ($NaHCO_3$, 2.0 equiv) in neat **8**. The reaction was executed at room

temperature with a noticeable increase in viscosity over time. Idesolide (**5**) began to crystallize in the reaction mixture as the conversion of **8** to idesolide (**5**) proceeded. The reaction took anywhere from 12 – 48 hours to surpass the ratio of 1:1 **8**/(**5**), as monitored by NMR, and rarely did the conversion of **8** ever surpass 75%. The purification of the reaction mixture consisted of trituration with pentanes or hexanes. (-)–Idesolide (**5**) was the only compound in the reaction mixture soluble in the non-polar solvent and yields were observed in the range of 56 – 66% using this method of isolation. The mixtures that contained (-)-idesolide (**5**) were not able to be isolated by chromatographic purification and attempts were detrimental to product recovery, despite other reports of its isolation by these means.

Although Kuwahara's conditions for the dimerization of **8** were consistent, a screen of solid support reagents was performed, Table 9. However, the conversion of **8** to (-)–idesolide (**5**) never exceeded 33% based on NMR analysis and a set of conditions superior to dispersed $NaHCO_3$ (s) was never established.

Table 9. Reagent screen – dimerization of **8** to (-)-idesolide (**5**).



(-)-**8** (-)-idesolide (**5**)

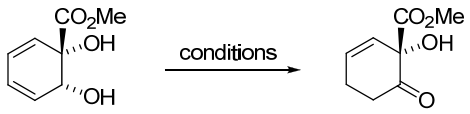
Entry	Solid Support	Result
1	Neutral alumina	Idesolide (**5**) formed, low conversion by TLC
2	Basic alumina (Brockman Act I)	Idesolide (**5**) formed, low conversion by TLC
3	Amberlyst A-21 Ion exchange resin (weakly basic)	Most promising by TLC; NMR conversion to idesolide (**5**) 33%
4	Amberlyst A-27 Ion exchange resin (strongly basic)	Idesolide (**5**) formed, low conversion by TLC

* 5 mg-scale of **8,** 100 wt% solid support, no solvent

Having established the means to produce the monomeric unit **8** of idesolide (**5**) the direct isomerization of diene diol **340** to **8** was studied, Table 10. Several metal catalyst are known for the isomerization of simple allylic alcohols.[243-247] Several sets of conditions were screened with diene diol **340** without successfully forming ketone **8**. Several reactive pathways exist in using transition metals with **340**: 1) a diene moiety, 2) doubly allylic alcohols, 3) *vic* diols, and 4) thermally labile substrate prone to re-aromatization *via* dehydration. In the course of the reagent screen, complex mixtures of

products were commonly encountered; however, two reactions stand out as their products were stable and isolable, Scheme 22.

Table 10. Reagent screen – attempts at the direct isomerization of **340** to **8**.



340 (-)-**8**

Entry	Conditions	Result
1	Crabtree catalyst $[Ir(cod)(PCy_3)(py)]PF_6$ (5 mol%), DCM	No reaction
2	$Fe_2(CO)_9$, DCM, hυ	Low conversion (4 spots) – **346** (35%, Scheme 22)
3	$Fe_3(CO)_{12}$, DCM, hυ	Low conversion (5 spots) – no **8**
4	$Fe_2(CO)_2$, PhMe, 45 ˚C	No reaction
5	TPAP, octanol, PhF, Δ	Complex mixture (5 spots) – no **8**
6	Grubbs I (5 mol%), toluene, Δ	Complex mixture (7 spots) – **347** (20%, Scheme 22)
7	$Fe(CO)_5$, DCM, hυ	Low conversion (3 spots) – no **8**
8	$Ru_3(CO)_{12}$, DCM, rt → 40 °C	Low conversion – decomposition
9	$Ru_2Cl_2(CO)_4$, DCM, rt → 40 °C	Low conversion (5 spots) – no **8**
10	$Ru_3(CO)_{12}$, DCM, rt → 130 °C, several days	Complex mixture (4 spots) – no **8**

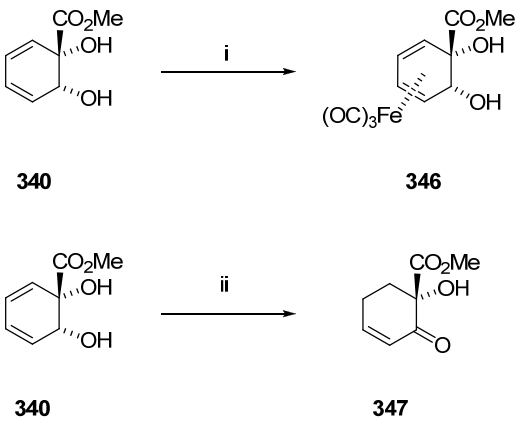
11	Pd/C, Et_3N, toluene, 130 °C	Complex mixture – no **8**, rearomatization (+ $FeCl_3$ test)
12	$RuCl_3 \cdot H_2O$, MeOH (rt, 16 h; 45 ˚C → 65 ˚C)	Non-polar spot (+ $FeCl_3$ test), low conversion, no **8**
13	$RuCl_2(PPh_3)_3$, MeOH (rt, 16 h; 45 ˚C → 65 ˚C)	Several non-polar spots formed upon heating, no **8**
14	$RhCl_3 \cdot 3H_2O$, MeOH (rt, 16 h; 45 ˚C → 65 ˚C)	Non-polar spot (+ $FeCl_3$ test), low conversion, no **8**
15	$[RhCl(C_2H_4)_2]_2$, MeOH (rt, 16 h; 45 ˚C → 65 ˚C)	No reaction

* 5 mg-scale **129**

Although not known to our research group at the time the group of Simon Lewis had been experimenting with iron(0) carbonyl complexes of diene diol **340** and reported the formation of η^4-cyclohexadiene complex **346**.[248] Lewis *was* able to form **346** in 55% yield over 15 days at room temperature using two equivalents of nonacarbonyldiiron [$Fe(CO)_9$]. Lewis reported the complete facial selectivity as being mediated by the hydroxy groups in **340**.[249] X-Ray analysis confirmed the structure of **346** and it was not until several months later that we could validate our structural assignment from his elucidation of iron(0) carbonyl complex **346**.

Allylic alcohol isomerizations using ruthenium catalysis have been documented, in particular TPAP[244] and Grubbs' catalyst[246] mediated isomerizations. Upon treatment of diene diol **340** with Grubbs' Generation I catalyst at elevated temperatures several reaction products formed. Non-polar material that tested positive with ferric chloride ($FeCl_3$) test, *i.e.* re-aromatized phenol, was observed and was in competition with **340-**

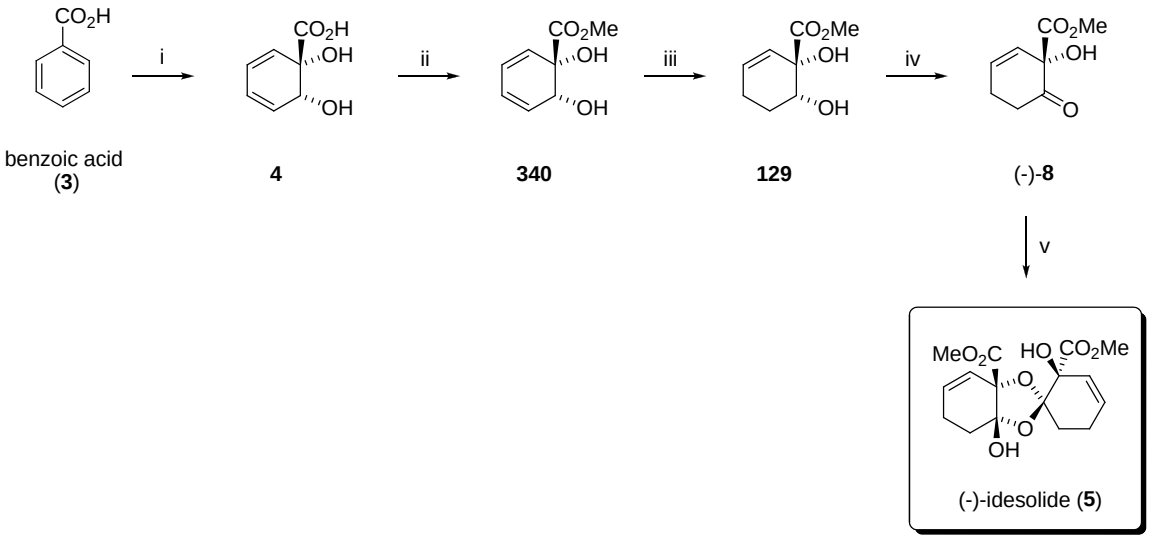
catalyst interaction. However, conversion of **340** to any product required elevation of temperature and enone **347** was isolated.



Reagents: (i) $Fe_3(CO)_{12}$, CH_2Cl_2, hυ, 3 h (35%); (ii) Grubbs' Generation I catalyst (5 mol%), PhMe, reflux, 1.5 h (21%).

Scheme 22. Conversion of diene diol **340** to iron carbonyl complex **346** and enone **347**.

Our inability to isomerize diene diol **340** was disappointing. However, the synthesis of (-)-idesolide (**5**) was concise in terms of step count. The overall yield of (-)-idesolide (**5**) from diene diol carboxylic acid **4**, in four chemical steps, ranged from 10 – 19% based on sets of average yields obtained at each step, Scheme 23.[70]



Reagents: (i) *R. eutrophus* B_9 (18 - 22 g/L); (ii) diazomethane, THF, 0 °C (70-76%); (iii) PAD, HOAc (glac), MeOH, 5 °C → rt (64%); (iv) IBX, DMSO, rt (60%); (v) $NaHCO_3$, neat, rt (66%).

Scheme 23. Chemoenzymatic synthesis of (-)-idesolide (**5**).

3.4 Chemoenzymatic Synthesis of Polyhydroxylated Pyrrolidines from Benzoic Acid

Many of the diene diol metabolites, *e.g.* **2**, derived from enzymatic dihydroxylation of aromatic compounds have been reported as part of the Hudlicky group biocatalysis program and have been used for the preparation of pseudo sugars,[250, 251] inositols,[252-254] and iminosugars.[255-257] The functional groups in **2** (1,3-diene, *vic* diol, vinyl halide, two different allylic alcohols) allow for many opportunities for redox transformations and different modes of oxygenation, Figure 51.

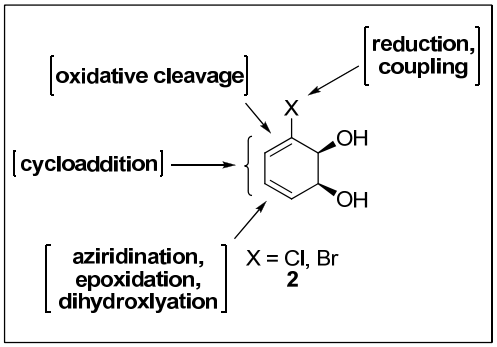


Figure 51. Functional handles in metabolite **2**.

However, the *ipso* diene diol **4** has not been utilized to the same extent as other halobenzene derive *cis*-dihydrodiols of type **2**. Recently, Parker[73] and Lewis[230] have prepared carbosugar derivative **94** and inositol-amino acid hybrids **348** – **350**, respectively, from benzoic acid, Figure 52.

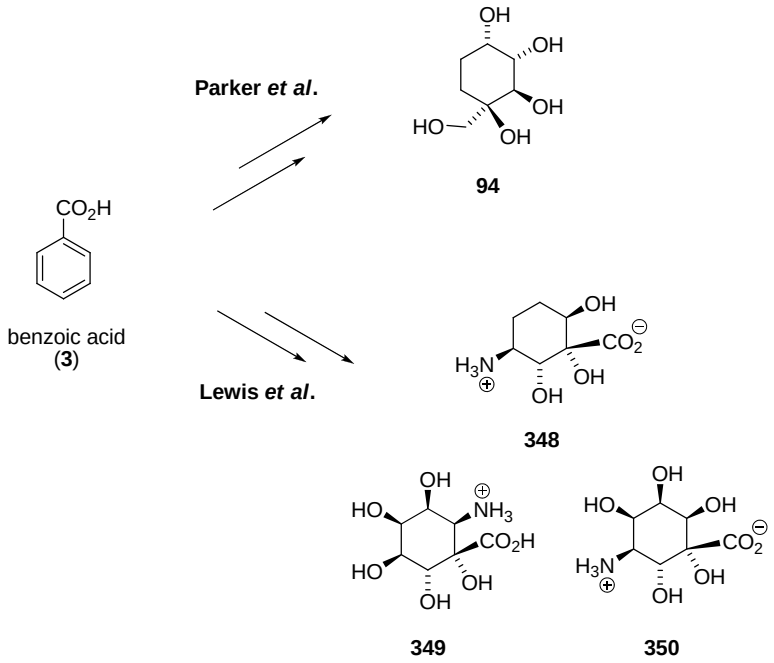


Figure 52. Carbosugar and inositol–amino acid derivatives.

3.4.1 Pyrrolidone Approach

In keeping with the theme of target oriented synthesis using *ipso* diene diol **4**, pyrrolidine **6**, an analogue of a recently reported iminosugar **151**,[119] was pursued on the basis of the highly oxygenated substitution pattern that contained a quaternary carbon center. Prior to the successful preparation of pyrrolidine **6** from diene diol **4** *via* a separate route and a set of intermediate compounds,[71] the synthetic route to pyrrolidone **351** was envisioned and was inspired by Danishefsky's vernolepin synthesis, Figure 53.[258] The

key transformation of this route was to be the oxidative cleavage and concomitant loss of a carbon atom from keto diol **352** to form diester **353** using lead tetraacetate [$Pb(OAc)_4$]. From intermediate **353** the deprotection of the *t*-butoxy carbonyl (Boc) group on the nitrogen would initiate the cyclization to the pyrrolidone skeleton of type **351**.

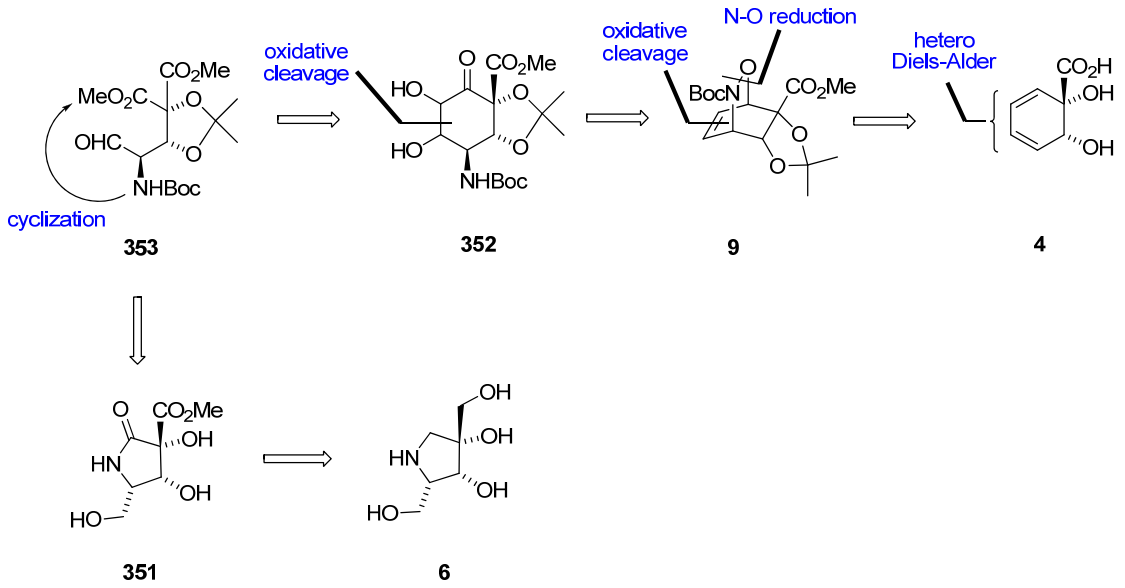
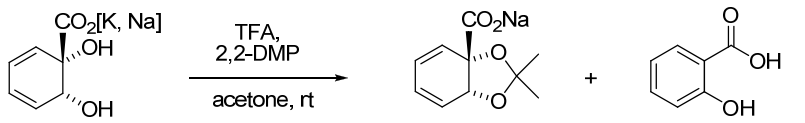


Figure 53. Retrosynthetic analysis of pyrrolidone **351**.

To this end, the *ipso* carboxylate diol **337**, isolated from the ferment broth as a mixture of sodium and potassium salts, was subjected to 2,2-dimethoxypropane (2,2-DMP, 1.0 M) and trifluoroacetic acid (TFA, 1.5 equiv) as a heterogeneous mixture in acetone (4.9 M) at 0 °C.[69] The formation of isopropylidene sodium carboxylate **354** was inconsistent, at best, in terms of impurity profile, conversion, and yield. A strong acid, TFA, was required to counteract the buffering effects of the inorganic phosphate salts carried over from the ferment broth. Protonation of the carboxylate **337** was required for acetalization to proceed. In using such a strong organic acid as TFA dehydration to salicyclic acid (**339**) was a major cause for impurities in the reaction mixture run-to-run. High pressure liquid chromatography (HPLC) was used to monitor the reaction and we

could easily separate carboxylate **337** from acetonide **354** and salicylic acid (**339**) by this method and provided relative percentages of each. The influence of TFA and 2,2-DMP on the reaction was evident from previous reactions where yields would vary from 30% – 60%. Full conversion of **337** required the titration of additional TFA (> 4 equiv) over 24 – 48 hours, and salicylic acid (**339**) was present in ranges of 20 – 30%. The inconsistency of reaction profiles and the low yields at the beginning stages of the material supply line prompted the statistical analysis of the formation of isopropylidene carboxylate **354** from carboxylate diol **337**. A two-level, three-factor design of experiment (2^3 DoE)[259] was performed using the mole ratio of TFA/**337**, concentration of 2,2-DMP, and concentration of acetone as the main effect variables along with interaction effects analyzed in the design (*i.e.* full factorial design), Table 11.

Table 11. 2^3 Full factorial DoE for the acetalization of **337**.



337 **354** salicylic acid (**339**)

Run	**TFA/337** (mol/mol)	**2,2-DMP** (mol/L)	**acetone** (mol/L)	**337** (%)	**354** (%)	**339** (%)
1	1.5	0.5	0	32.5	63.3	4.2
2	1.5	0.5	6	25	70.2	4.8
3	1.5	2	0	20.1	72.1	7.8
4	1.5	2	6	21.8	73.7	4.5
5	6	0.5	0	7.2	80.4	12.4
6	6	0.5	6	7.4	79.4	13.2
7	6	2	0	7.5	71.3	21.2
8	6	2	6	5.7	74.0	20.3

* **337** (purity of 76 wt% based on NMR internal standard method)
* Reactions performed in parallel, 100 mg-scale
* Percentages based on HPLC area under the curve (AUC), non-calibrated response factors.

By utilizing the multivariate analysis software SAS JMP® 10.0.0, main effects and interaction effects were identified and modeled (see plot, Figure 54). The largest main variable effect was the mole ratio of TFA to **337**, a 17-fold larger effect than the next largest main effect – the concentration of 2,2-DMP. A large interaction effect between TFA/**337** mole ratio and the concentration 2,2-DMP exists for this reaction. Using the data generated from the DoE screen, the reaction was performed two additional times at increasingly larger scales. The reaction was performed on 4.5 g-scale (79% yield) and 61.6 g-scale [90% yield, < 4% salicylic acid (**339**)] using 6.0 equivalents of TFA, 0.4 M 2,2-DMP, and no acetone at 0 ˚C. These conditions represent the optimal reaction parameters for substrate **337** of that particular chemical composition, 79% purity that contained inorganic phosphate salts used as buffers in the fermentation of benzoic acid. The material from the fermentation, isolated in the same way as previously described, did not vary significantly in terms of purity and the optimized reaction conditions served well for diol **337** of similar weight-weight composition. The reaction was complete in approximately 12 hours and allowed for ample acetonide **354** (multiple 100 gram-scale batches) to be obtained for the subsequent synthetic work.

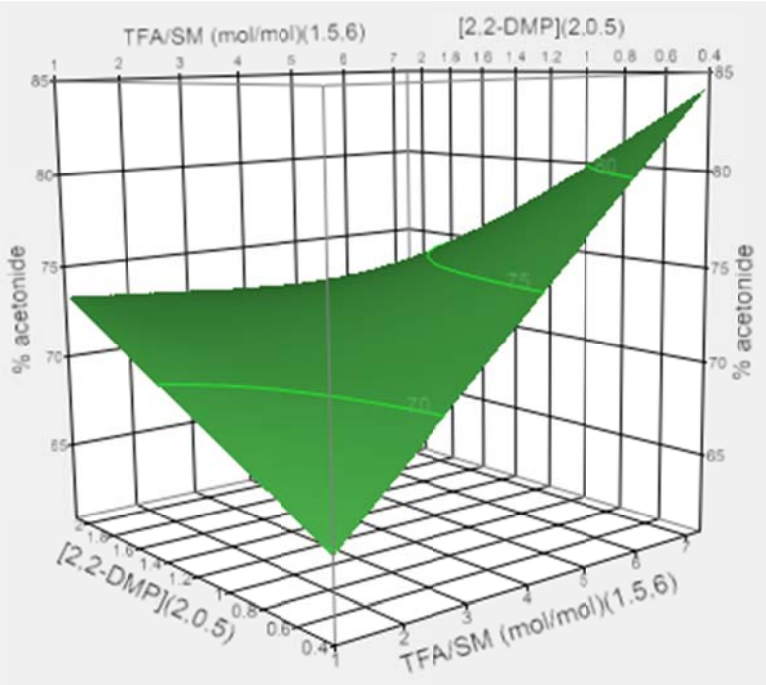
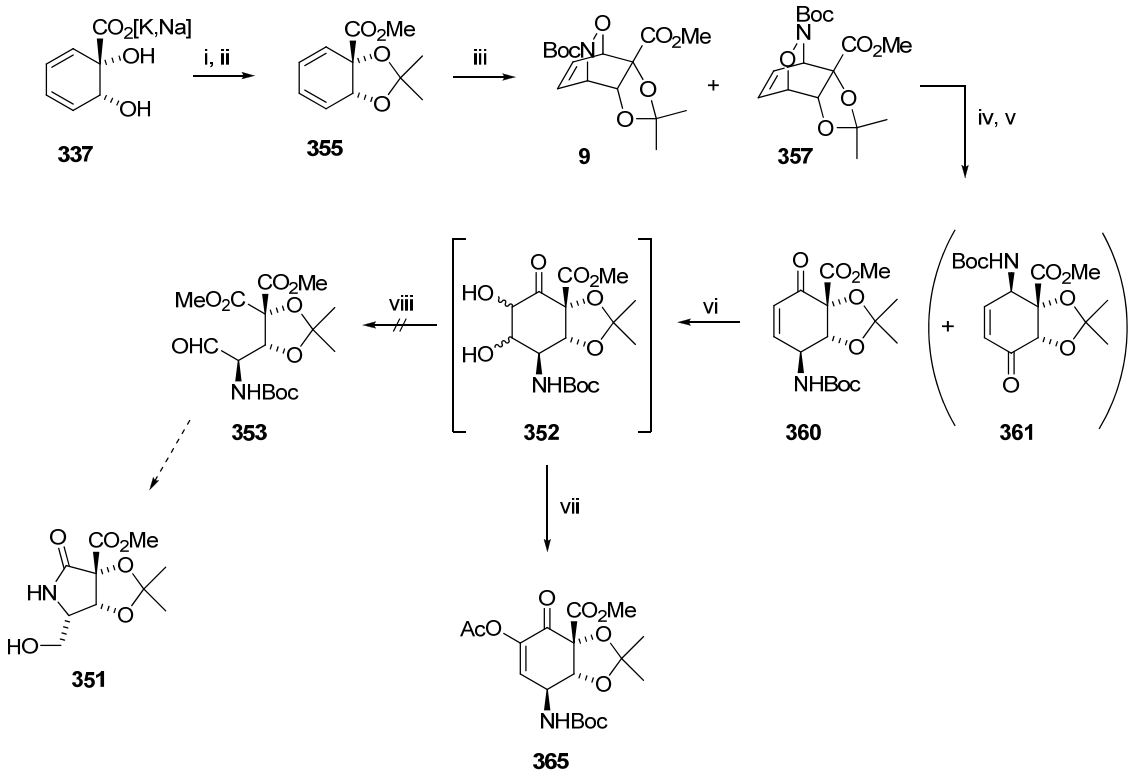


Figure 54. Fitted model for yield of acetonide **354**.

The preparation of sodium carboxylate **354** was followed by the methylation to methyl ester **355**, Scheme 24. Two sets of reaction conditions were used to form methyl ester **355** and depended on the scale of the reaction. First, and prior to methylation of carboxylate **354**, protonation of the carboxylate in an aqueous solution was performed at 0 ˚C followed by extraction and concentration *in vacuo*. This was done quickly and storage of the carboxylic acid acetonide **356** was avoided. Small scale methylation reactions were performed using an etheral solution of diazomethane generated in a biphasic system consisting of an alkaline solution of *N*-nitrosomethyl urea and diethyl ether at 0 ˚C.[260] Larger scale reactions, which were more typical at this stage, were completed using carbodiimide and MeOH. The diene in methyl ester **355** was transformed to hetero Diels-Alder adducts **9**/**357** (6:1 ratio) by the generation of *N*-acyl nitroso dienophile derived from the *in situ* oxidation of *N*-Boc hydroxyl amine by sodium periodate ($NaIO_4$). Crude yields of adducts **9**/**357** were good (96%) after filtration of the

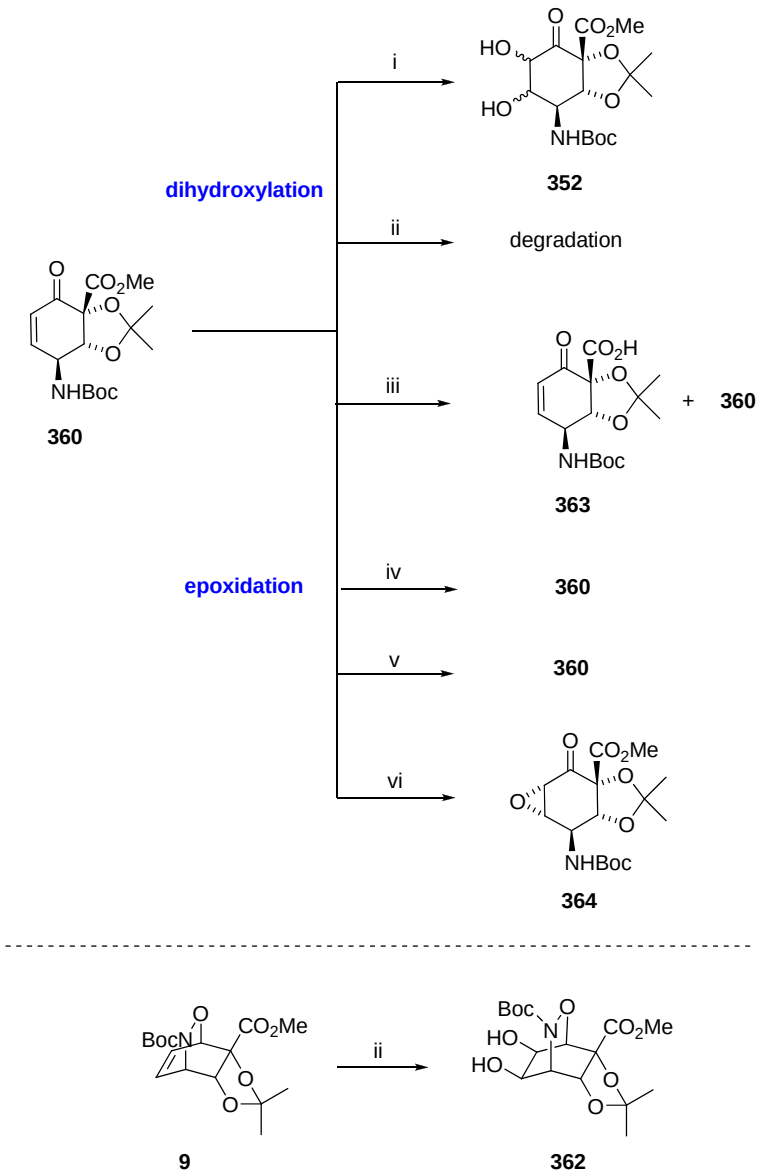
reaction mixture and an aqueous base wash ($NaHCO_3$). The regioisomers were not separable by physical or chemical means and the ratio of isomers was determined by NMR. Treatment of **9/357** with molybdenum hexacarbonyl [$Mo(CO)_6$] in aqueous acetonitrile (MeCN) caused the reduction of the nitrogen – oxygen bond to amino alcohols **358/359** (not shown).[261] This mixture was further subjected to oxidation with Dess-Martin periodinane (DMP) to yield a separable mixture of enones **360** and **361**.



Reagents: (i) 2,2-DMP, TFA, 0 °C (90%); (ii) DCC, DMAP, MeOH, CH_2Cl_2, 10 °C (67%); (iii) *t*-butyl hydroxycarbamate, $NaIO_4$, MeOH/H_2O (4:1 v/v) (96% crude yield; 6:1 **9/357**); (iv) $Mo(CO)_6$, MeCN/H_2O (15:1 v/v), 80 °C; (v) Dess-Martin periodinane, CH_2Cl_2, 0 °C (32% from **9/357**); (vi) $NaIO_4$, $CeCl_3{\cdot}7H_2O$, $RuCl_3{\cdot}xH_2O$, MeCN/H_2O/EtOAc, rt (quant); (vii) Ac_2O, DMAP, Et_3N, CH_2Cl_2 (52%); (viii) $Pb(OAc)_4$, MeOH/PhH (1:1 v/v).

Scheme 24. Synthetic route to pyrrolidone **351**.

Standard Upjohn dihydroxylation conditions[262] were problematic for the electron deficient enone **360** and yielded complex mixtures. A couple of different strategies were attempted with only marginal success, such as epoxidation (products such as **362** and **363**) and hydroxylation of **9** to give **364**. Plietker and co-workers had developed a bimetallic $RuCl_3$–Ce(IV)–periodate system, prepared by heating an aqueous mixture of cerium chloride heptahydrate ($CeCl_3 \cdot 7H_2O$) and $NaIO_4$ and mixing with ruthenium chloride ($RuCl_3$), that was mild enough not to undergo glycol cleavage typical of periodate oxidations and diol **352** was formed, Scheme 24 and 25.



Reagents: (i) $NaIO_4$, $CeCl_3{\cdot}7H_2O$, $RuCl_3{\cdot}xH_2O$, MeCN/H_2O/EtOAc, rt (quant); (ii) OsO_4, NMO, acetone/H_2O (4:1 v/v) (90% for **362**); (iii) H_2O_2 (aq, 30%), NaOH (cat.) MeOH; (iv) *t*BuOOH, $VO(acac)_2$ (10 mol%), CH_2Cl_2; (v) *m*CPBA, CH_2Cl_2, 0 °C → rt; (vi) *t*BuOOH, Triton B, THF, rt (33%).

Scheme 25. Oxidation of enone **360**.

The stability of **352** was suspect because it underwent elimination to form acetoxy enone **365** upon derivatization with Ac_2O, Scheme 24. The conditions presented in the literature, and slight alterations thereof, were attempted on keto diol **352**.[258, 263] The combined lability of **352** and the aggressiveness of $Pb(OAc)_4$ produced unidentifiable

mixtures immediately upon addition and indicated by a deep red color. No evidence of diester aldehyde **353** was ever obtained and no further extrapolation to pyrrolidone **351** was pursued. At this point a secondary synthetic strategy was assessed.

3.4.2 Pyrrolidine Approach

Having met with failure with the synthesis of pyrrolidone **351** a secondary strategy was implemented that would provide pyrrolidines of type **6** *via* acetal **11**, Figure 55.

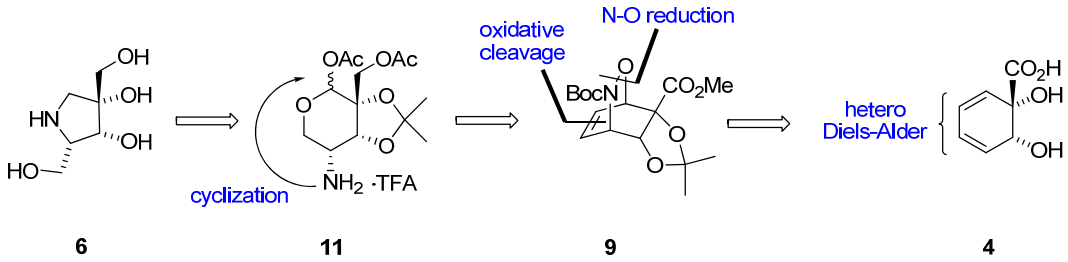
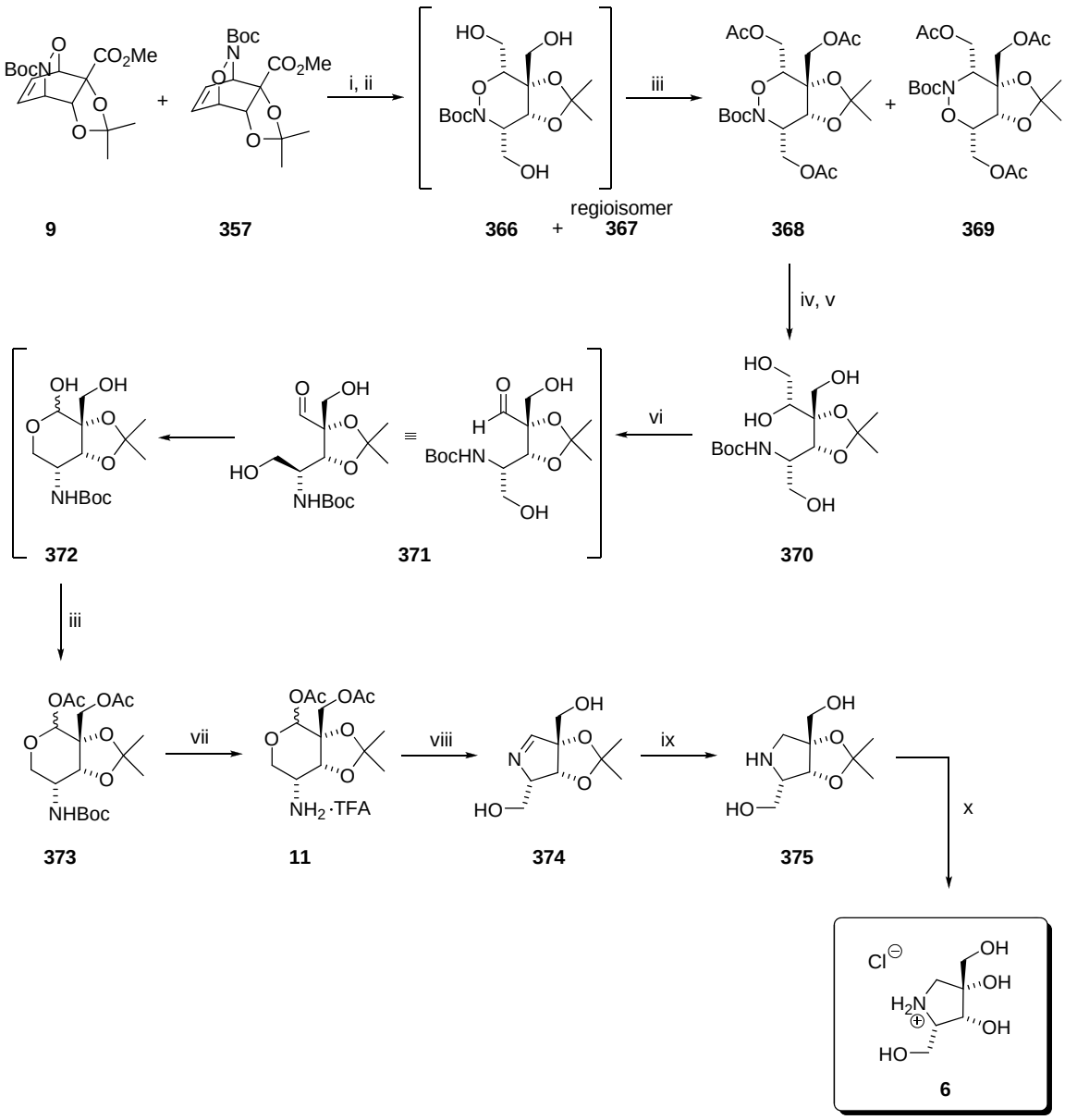


Figure 55. Retrosynthetic analysis of pyrrolidine **6**.

The synthesis was redisgned and initiated from isoxazolidines **9**/**357** and was subjected to ozonolysis in order to "unfold" the molecule *via* oxidative cleavage and add necessary oxygenation for subsequent functional maneuvers, *i.e.* the formation of acetal **11** *via* cyclization, Scheme 26. The intermediate ozonide of **9**/**357** was reduced with $NaBH_4$ at -55 ˚C with slow warming to room temperature over several hours (> 8 h) to yield an intermediate triol **366**/**367**. This highly polar material, like several subsequent intermediates in this approach, was trapped as triacetate **368**, which was isolated by column chromatography in yields ranging from 22 – 37% from the 6:1 mixture of **9**/**357**. The primary function of the acetylation of triol **366**/**367**, other than to cap the polar functional groups, was to separate the regioisomeric mixture of **368**/**369**. Having isolated

368, the acetates were removed by methanolysis to give **366** and the nitrogen – oxygen bond was reduced using $Mo(CO)_6$ to give tetrol **370** that contained a set of vicinal 1,2-diol moieties. The N–O bond cleavage was a difficult reaction at first and alternative conditions, such as hydrogenolysis and mercury – aluminum amalgam, were inert toward substrate **366**. A brief study of this reaction indicated that the purity of the $Mo(CO)_6$ was of importance in achieving homogeneity of the reaction mixture. To this end $Mo(CO)_6$ was purified by sublimation. Another crucial observation that was made indicated that the formation of the active species $Mo(CO)_3(MeCN)_3$ that could be observed by TLC throughout the reaction.[264, 265] $Mo(CO)_3(MeCN)_3$ was thus prepared, in an unknown purity, and was shown to reduce the N–O bond in substrate **366** in MeOH at reflux in significantly less time, less than one hour compared to greater than ten hours at reflux in $MeCN/H_2O$. However, there was some variation in subsequent reactions that was attributed to the purity and/or stability of the $Mo(CO)_3(MeCN)_3$ species. Last, and most significant, was the workup for the molybdenum reduction. Effective decomplexation of the product **370**-molybdenum species was completed by the addition of sodium bicarbonate (either solid $NaHCO_3$ or as a saturated solution) and vigorous stirring as to introduce air into the reaction mixture. This method of workup turned the black and viscous reaction mixture that contained **370** into a light brown heterogeneous mixture that contained finely dispersed precipitate in twelve hours. Yields up to 87% were achieved for this reaction with the improved workup procedure. Now, that the 1,2-diol moiety had been unmasked, all that was left in order to access acetal **11**, the precursor to pyrrolidine **6**, was to oxidatively cleave the glycolic bond in **370** to form aldehyde **371** and trap the resulting hemiacetal **372** as acetal **373**. Initial conditions for the reaction of **370** to **372**

included a biphasic system of aqueous alkaline solution of $NaIO_4$ and methylene chloride. Hemiacetal **372** was difficult to capture in a pure state under these conditions and the aldehyde signals in NMR spectrum were typically observed in varying degrees. An excess of alkali was probably suppressing equilibria and so another method was sought. Silica gel–supported periodate[266] provided a much cleaner reaction profile and workup consisted of the filtration of the reaction mixture that contained hemiacetal **372** directly into another mixture of $Ac_2O/Et_3N/DMAP/CH_2Cl_2$. The telescoped reaction sequence provided acetal **373** in 41% yield from tetraacetate **368**. All that was left for the final cyclization to the pyrrolidine core was the deprotection of the strategically placed protecting groups. Acid-mediated decomposition of the *tert*-butoxy carbonyl (Boc) group with strong organic acid (TFA) provided the amine TFA salt **11** which, upon treatment with basic methanol, unmasked the electrophilic hemiacetal and concomitantly cyclized, *via* neutralization of the amine salt **11**, to generate azomethine **374**. The hydrogenation of **374** using palladium on charcoal (Pd/C) and subsequent hydrolysis of the isopropylidene yielded **375** and final iminosugar **6**, respectively, and completed the synthesis of the highly oxygenated pyrrolidine ring system.



Reagents: (i) O_3, CH_2Cl_2, -70 °C; (ii) $NaBH_4$, -55 °C → rt; (iii) Ac_2O, Et_3N, DMAP, CH_2Cl_2, rt (for **368**: 22% from **9**/**357**; for **373**: 56% from **370**); (iv) K_2CO_2 (aq, 10%), MeOH (quant); (v) $Mo(CO)_6$, MeCN/H_2O (15:1 v/v), reflux (87%); (vi) SiO_2-$NaIO_4$ (15% w/w), CH_2Cl_2; (vii) TFA, CH_2Cl_2, 0 °C → rt (62%); (viii) K_2CO_3(s), MeOH (anhydrous) (81%); (ix) Pd/C, H_2 (1 atm), HOAc (aq) (quant); (x) HCl (conc), MeOH, 40 °C (75%).

Scheme 26. Synthesis of pyrrolidine **6**.

The inspiration for the synthesis of pyrrolidine **6** came from a report describing a series of iminosugars prepared by BioCryst in 2011 in their effort to identify a more

potent inhibitor of human purine nucleoside phosphorylase (PNP).[119] In addition to forming five-membered heterocyclic compounds from *ipso* diol **4**, a series of diastereomers, with respect to **151**/**209**, that contained an additional oxygen atom was envisioned and included compounds **6** and **376**, Figure 56.

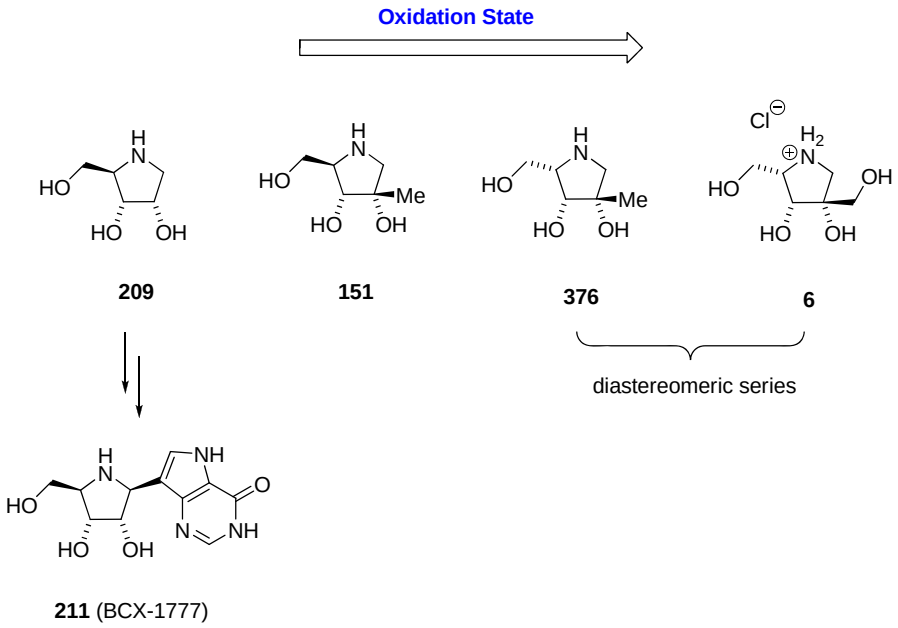
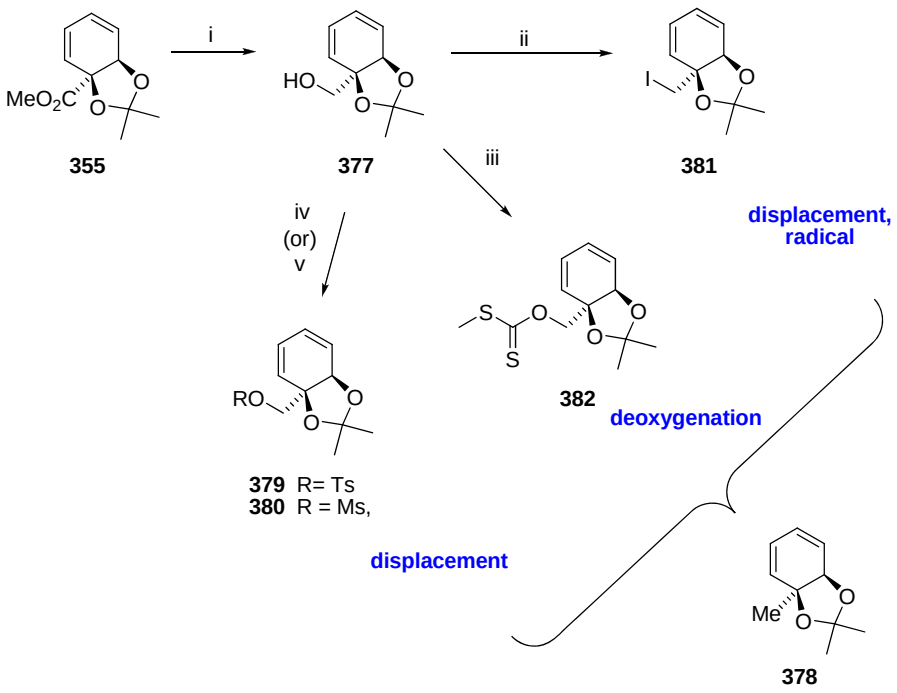


Figure 56. Pyrrolidine series.

Having prepared tetra hydroxylated pyrrolidine **6** we sought to reduce the hydroxy methylene attached to the quaternary center in **6**. In doing so, the reduction of an early intermediate in the synthesis toward **376** was attempted, diene ester **355**. Diene ester **355** was used to provide diene alcohol **377** *via* hydride reduction with lithium borohydride ($LiBH_4$) and the different tactics used in the attempts to reduce the primary alcohol **377** to **378** are shown below, Scheme 27.

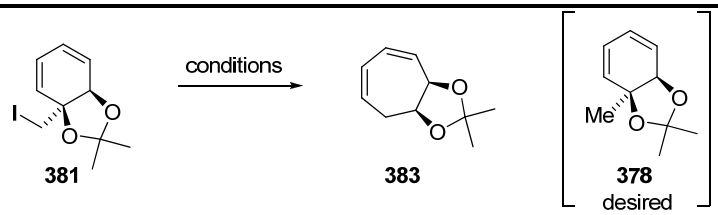


Reagents: (i) $LiBH_4$, THF, 0 → rt (83%); (ii) Ph_3P, I_2, PhMe (23%); (iii) CS_2, KHMDS, THF, -60 °C; then MeI (82%); (iv) for **379**: *p*TsCl, Et_3N, CH_2Cl_2, 0 °C → rt (71%); (v) for **380**: MsCl, Et_3N, CH_2Cl_2, 0 °C → rt (89%).

Scheme 27. Reduction of **377** attempts.

The displacement of iodide **381** was attempted using the conditions listed below, Table 11. The ring expansion of **381** was observed under radical conditions (entry 6) *via* cyclopropane; however, no reduction to **378** was observed.

Table 12. Reduction of iodide **381**.

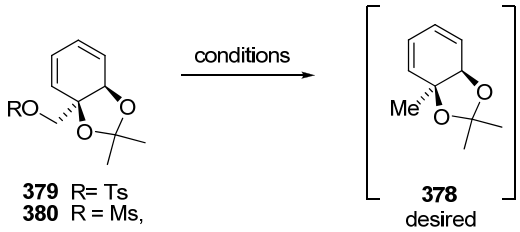


381 **383** **378** desired

Entry	Conditions	Result
1	NaBH4, DMSO, 80 ˚C	no reaction
2	$LiEt_3BH$, DMSO, RT → 80 ˚C	**381** + trace polar material
3	$LiEt_3BH$, THF, reflux	no reaction
4	KH, 18-C-6, DME, reflux	no reaction
5	$LiAlH_4$, THF, RT → reflux	complex mixture
6	$n Bu_3SnH$, AIBN, PhH, reflux	complex mixture; trace **383**

Further attempts at the displacement of tosylate **379** and mesylate **380** with hydride sources failed to provide **378**, Table 13.

Table 13. Sulfonate **379**/**380** displacement attempts



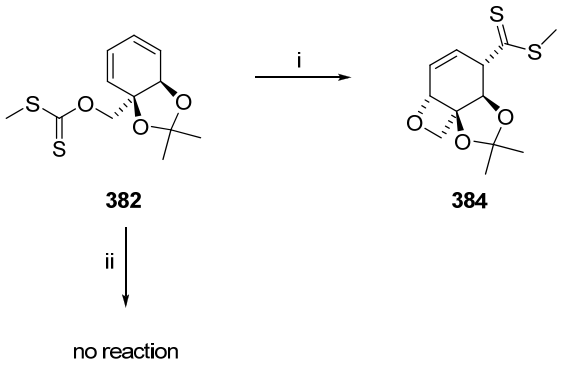
379 R= Ts
380 R = Ms,

378
desired

Entry	Conditions	Result
1	**379**: $LiAlH_4$, THF, -50 °C → 0 °C → RT	no reaction, **379** (85%)
2	**379**: $NaBH_4$, 15-Crown-5, DME, 0 °C → RT	no reaction, **379**
3	**379**: $LiBH_4$, 12-Crown-4, DME, 0 °C → RT	no reaction, **379**
4	**379**: $LiAlH_4$, THF, reflux	no reaction, **379**
5	**380**: $LiAlH_4$, THF, 0 °C → RT	aromatization
6	**380**: $LiBH_4$, DME, 0 °C → RT, >48 h	no reaction, **380**

The lability of primary xanthates and their tendency to undergo a slow fragmentation step to an unstable primary radical during the Barton-McCombie deoxygenation reaction provides the opportunity for substrates to enter into competition with other reaction pathways, *e.g.* formation of thioformates, xanthate transfer reactions, *etc*.[267] Xanthate **382** was prepared in good yield (82%) by using a non-traditional base, potassium hexamethyldisilazide (KHMDS), and low temperatures (-60 ˚C). The use of NaH at and above room temperature in the formation of xanthate **382** provided only traces of the desired product (3%). The treatment of **382** under thermally initiated radical conditions ($n$$Bu_3SnH$, AIBN) provided oxetane **384** as the only identifiable compound of a complex mixture, Scheme 28. Triethylborane/air/water was also used to no avail in the formation and subsequent trapping the primary radical with a hydrogen atom.[268] The

deoxygenation of primary alcohol **377** was never realized with the methods and intermediates utilized by this practitioner.



Reagents: (i) nBu$_3$SnH, AIBN, PhH, reflux (5%); (ii) Et$_3$B, H$_2$O, PhH, rt.

Scheme 28. Deoxygenation of **382** attempts.

The synthesis of pyrrolidine **6** and its precursors demonstrate the utility of *ipso* diol **4** as a useful raw material for a variety of reactive intermediates. The synthesis of iminosugar **6** was completed in 13 chemical steps that included a hetero Diels-Alder reaction, two oxidative scissions, numerous redox operations, two cyclizations, and provided an opportunity to orchestrate different modes of those cyclization based on orthogonal protecting group strategies (nitrogen *vs.* oxygen and 1˚ alcohols *vs.* 1,2-diol).

3.5 Approaches to Synthesis of Vinca Alkaloids

This chapter will discuss the various approaches toward the tricyclic core of type **15**, the central core of aspidosperma alkaloids, and include a thermally induced 1-aza diene approach using **385**, an amidyl radical cyclization approach using **386**, miscellaneous cycloaddition and indolization approaches, and a tandem conjugate addition approach using intermediates **387** and **388**, Figure 57.

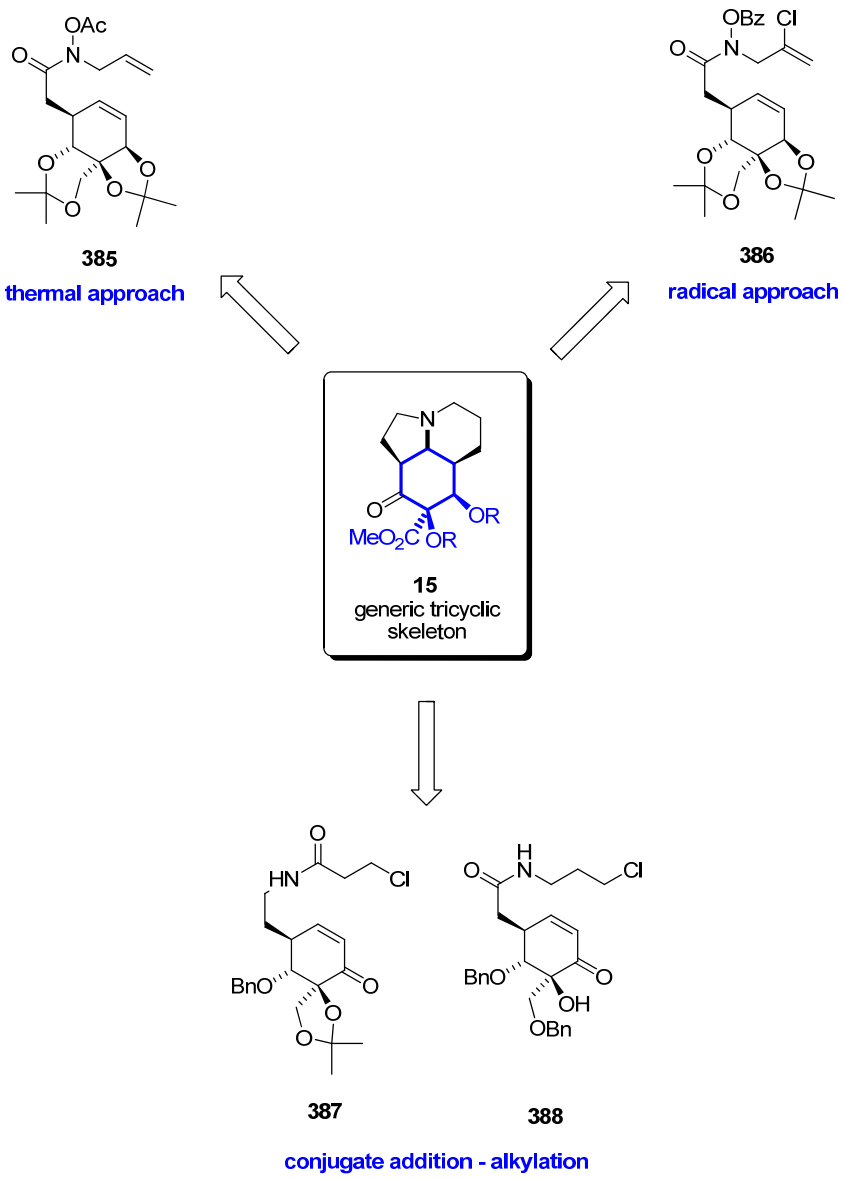


Figure 57. Overview of synthetic approaches towrd tricycle **15**.

3.5.1 Thermally Induced 1-Aza-1,3-Diene Diels-Alder Approach

1-Aza-butadienes have been used in the construction of six membered heterocycles since at least the early 1980's when Ghosez[269] and Fowler[270] reported on the thermally induced cycloadditions of various pre–functionalized substrates.

The presence of a nitrogen atom in aza-butadienes effectively lowers the highest occupied molecular orbital (HOMO) energy in comparison to non-heteroatom containing butadienes. The energy difference between the lowest unoccupied molecular orbital (LUMO) of electron deficient dienophiles is increased by the presence of the nitrogen in the diene and makes the cycloaddition reaction more difficult to perform. Therefore, 1-aza-butadienes have been utilized in inverse–demand Diels–Alder reactions where an electron rich dienophile is used to bridge the HOMO – LUMO energy gap, Figure 58.

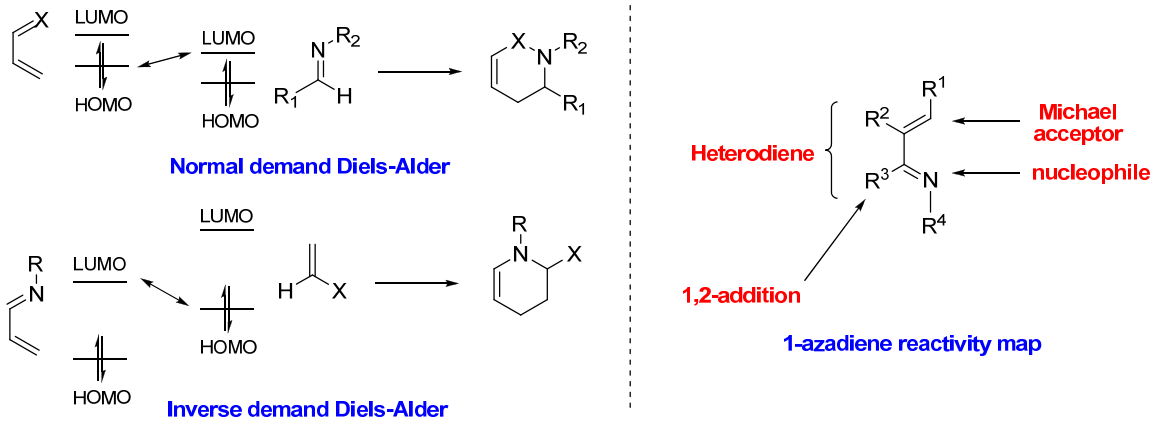
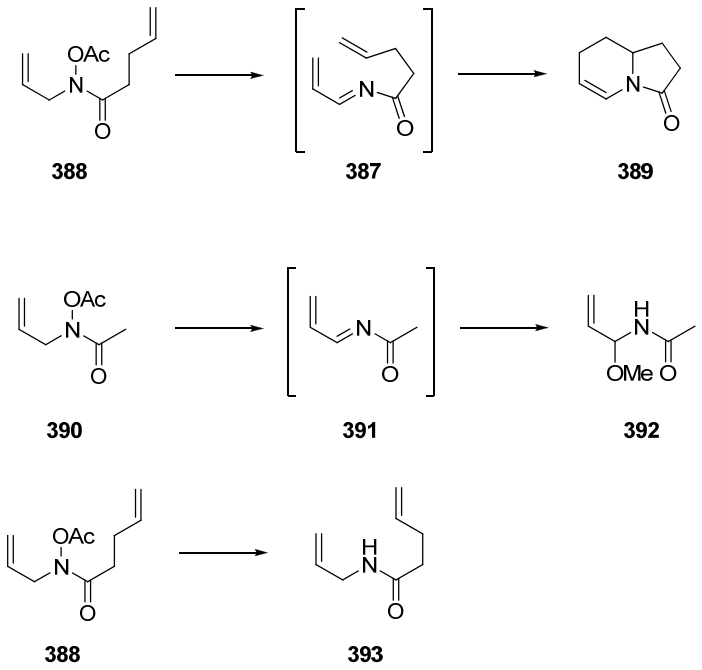


Figure 58. Azadiene HOMO-LUMO and reactivity map.

Conversely, many reports are available where electron deficient 1-aza-butadienes such as 2-cyano-1-aza-butadiene[271-276] or *N*-sulfonyl-1-aza-butadiene[277, 278] have been used to perform cycloaddition reactions with unactivated alkenes. Recently, catalytic versions of the [4+2]-cycloaddition reaction involving 1-aza-dienes have been developed

and offer good enantioselectivity.[279] The main strategy in the development of the enantioselective variants has focused on the activation of 1-aza-dienes through lowering of the LUMO energy by Lewis acid metal complexes and organocatalysis.

Much of the work presented here is based on the early reports by Fowler where he constructed a series of bicyclic heterocycles from linear 1-aza-butadienes. The 1-aza-butadienes, such as **387**, were formed thermally and in the gas phase by loss of acetic acid from *O*-acetylhydroxamic acids of type **388** and yielded indolizidines such as **389**, Scheme 29.[270] Evidence of 1-aza-butadiene formation was provided from a methanol quench where a benzene-d_6 solution of hydroxamic acid **390** was passed through a hot reaction tube with methanol placed in the receiving flask. In this fashion was the acyl imine **391** briefly formed and trapped as amide **392**. When Fowler attempted to form 1-azadiene of **388** in toluene at 180 ˚C for several hours the loss of acetic acid was observed but only amide **393** was obtained from the reaction.



Reagents: (i) 650 °C, pyrolysis tube (75%); (ii) 650 °C, pyrolysis tube, MeOH trap (69%); (iii) PhMe, 180 °C, 20 h (no yield reported).

Scheme 29. Pyrolysis of *O*-acetylhydroxamic acids to form 1-aza-butadienes.

Intrigued by Fowler's work and the desire to efficiently construct the tricyclic ring system of type **394**, we focused on obtaining intermediate **385** on the assumption of gaining entropic advantage for the cyclization through the loss of acetic acid. The path to the key hydroxamic intermediate **385** is outlined below and proceeds through the epoxy ester **395** and eventually to coupling partners **396** and carboxylic acid **397**, Figure 59.

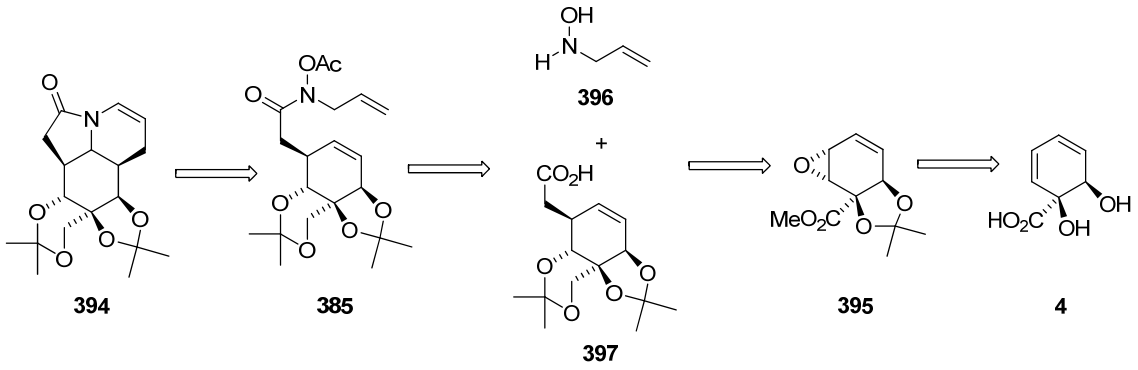
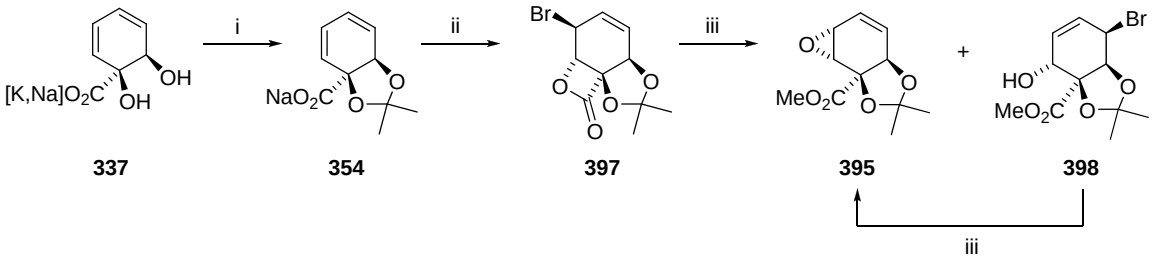


Figure 59. Synthetic analysis for tricycle **394**.

The *ipso*-diol carboxylate **337** was protected as acetonide sodium carboxylate **354.** An alkaline solution of **354** was treated with a solution of bromine in methylene chloride at 0 ˚C to afford bromo lactone **397** as a single regioisomer, Scheme 30.[66, 280] The treatment of bromo lactone **397** with anhydrous sodium methoxide (NaOMe) gave a mixture of epoxy ester **395** and allylic bromide **398** in approximately 1:1 ratio. Isolation of **398** and resubjection to NaOMe would produce another mixture that contained more of epoxide **395**. In this fashion the epoxy ester **395** could be produced.

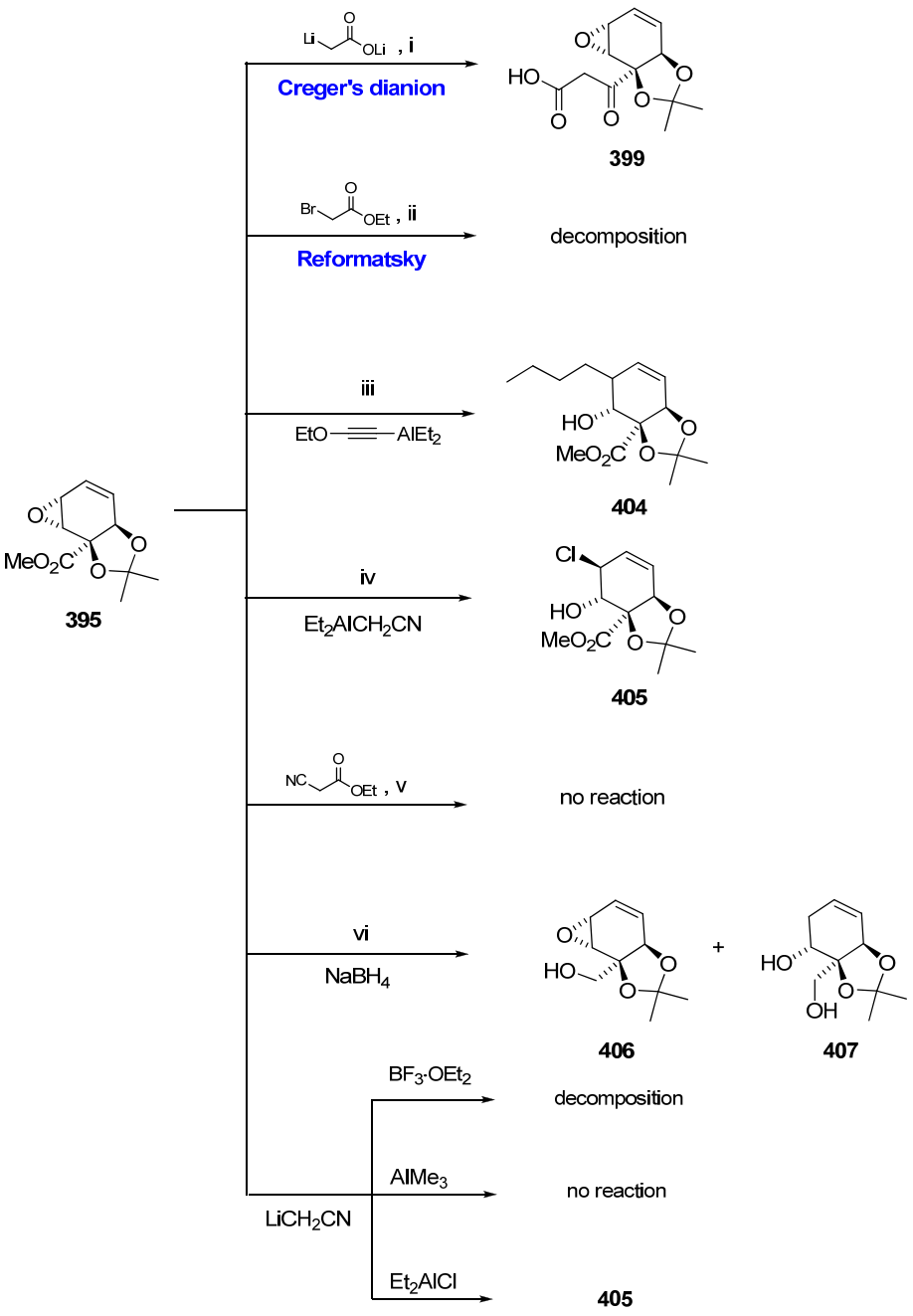


Reagents: (i) 2,2-DMP, TFA, 0 °C → rt (94%); (ii) Br_2, $NaHCO_3$ (aq), CH_2Cl_2, 0 °C → rt (30%); (iii) NaOMe, MeOH, 0 °C (58%).

Scheme 30. Synthesis of epoxy ester **395**.

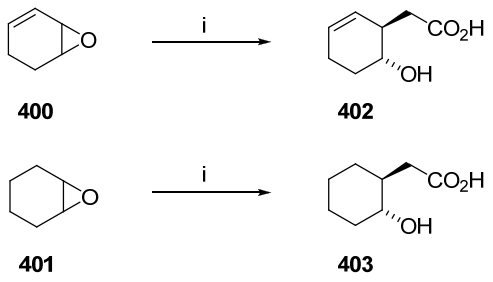
The selective opening of the epoxide ring in the epoxy ester **395** with a two-carbon nucleophile was attempted. However, despite the reaction conditions and additives that were used the electrophilicity of the ester in **395** outweighed that of the

epoxide. No two-carbon nucleophiles could be added to the epoxide in the presence of the ester, Scheme 31. Creger's dianion of acetic acid,[281] used by Danishefsky in his synthesis of vernolepin,[258] was unsuccessful and produced 1,3-dicarbonyl **399**, despite successful epoxide openings on model systems **400** and **401**, Scheme 32. Organo-aluminum compounds did not show any preference in delivering the anionic ligand and **404** and chloride **405** were formed from epoxide **395**. Sodium borohydride readily reduced the methyl ester to provide **406** and only upon heating the reaction mixture above room temperature did the epoxide in **406** open to furnish diol **407**. Lewis acid-mediated epoxide opening using the anion of acetonitrile also failed in the presence of the methyl ester of **395**.



Reagents: (i) $LiCH_2CO_2Li$, DME, 55 °C (40%); (ii) Zn, ethyl 2-bromoacetate, THF, 60 °C; (iii) ethoxyethyne, *n*BuLi, Et_2AlCl, PhMe, -55 °C (73%); (iv) MeCN, *n*BuLi, Et_2AlCl, PhMe, -65 °C; (v) ethyl 2-cyanoacetate, NaH, THF, 0 °C; (vi) $NaBH_4$, MeOH, 0 °C (75%); (vii) MeCN, *n*BuLi, THF, 0 °C, Lewis acid.

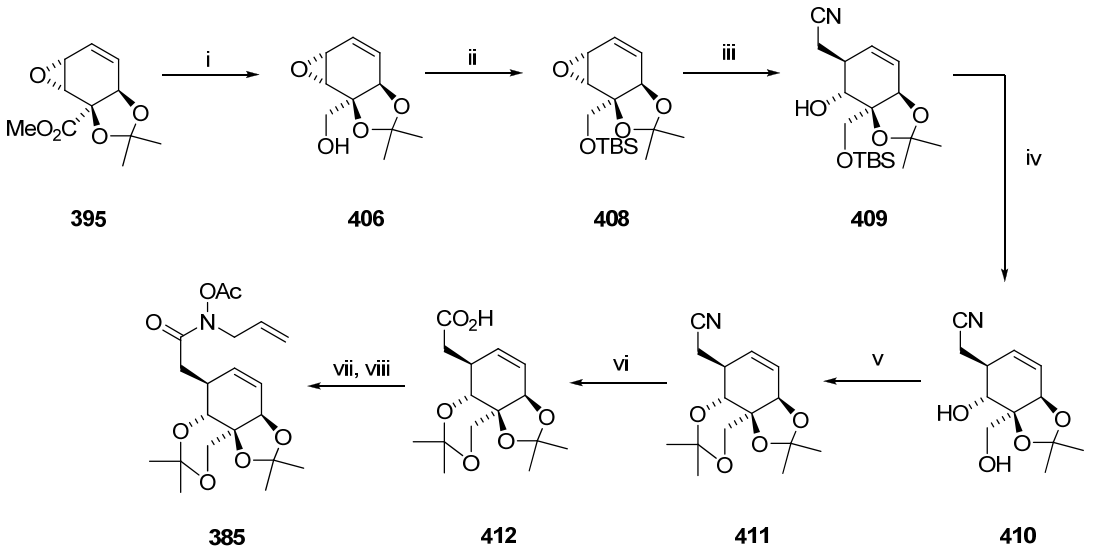
Scheme 31. Epoxide opening attempts of epoxy ester **395**.



Reagents: (i) $LiCH_2CO_2Li$, DME, rt.

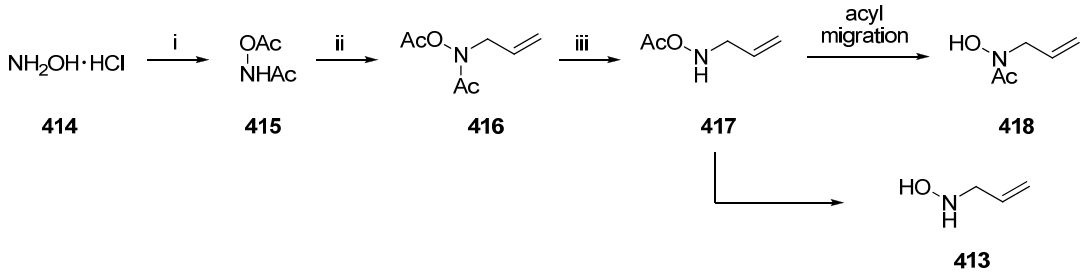
Scheme 32. Creger's acetic acid dianion opening of model epoxides **400** and **401**.

The electrophilic sink in epoxy ester **395** was removed by reduction to the primary alcohol **406**. The alcohol **406** was temporarily protected as the silyl ether **408** and the epoxide was readily opened with the anion of acetonitrile to yield cyano alcohol **409**, Scheme 33. Deprotection of **409** with fluoride gave diol **410** that was subsequently re-protected as the acetonide **411**. Saponification of cyano **411** gave carboxylic acid **412** after neutralization. The coupling of carboxylic acid **412** with *N*-allyl hydroxylamine **413**, prepared from hydroxylamine hydrochloride (**414**), Scheme 34)[270, 282, 283] yielded hydroxamic acid **385** after acylation with acetic anhydride.



Reagents: (i) $NaBH_4$, MeOH, 0 °C (75%); (ii) TBSCl, imidazole, DMF, rt (97% crude); (iii) MeCN, *n*BuLi, THF -52 °C → -10 °C (85%); (iv) TBAF, THF, rt (72%); (v) 2,2-DMP, *p*TsOH, acetone, (98%); (vi) NaOH (aq, 6 M), MeOH, reflux (97% crude); (vii) isobutylchloroformate, **413**, Et_3N, THF, 0 °C (56%); (viii) Ac_2O, DMAP, Et_3N, CH_2Cl_2 (quant).

Scheme 33. Synthesis of hydroxamic acid **385**.

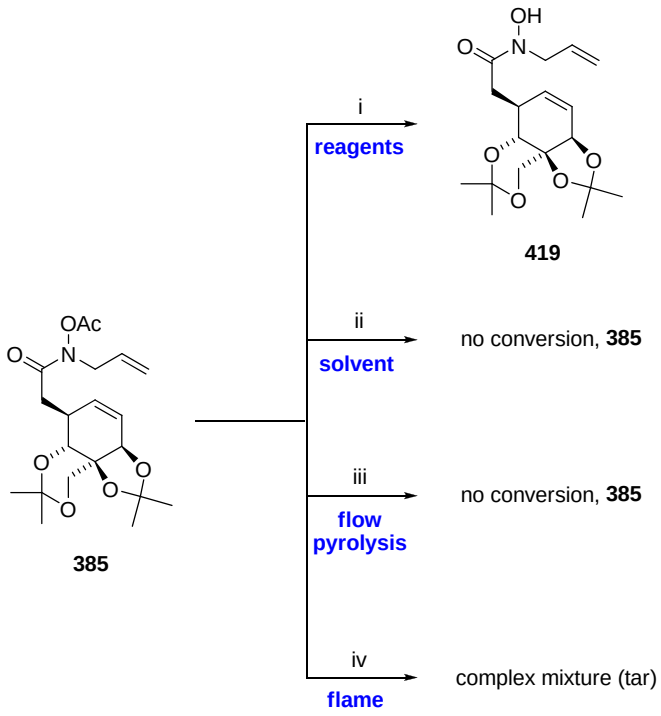


Reagents: (i) Ac_2O, NaOAc, reflux, 20 min (40%); (ii) allyl bromide, K_2CO_3, DMF, 24 h (46%); (iii) HCl (aq, 6 M), 1 h; (iv) HCl (aq, 3 M), reflux, 1.5 h (86%).

Scheme 34. Synthesis of *N*-allyl hydroxyl amine **413**.

Initially, O-acyl hydroxamic acid **385** was investigated in attempts to trap the acylimine (1-azadiene) with MeOH upon treatment with potassium *t*-butoxide (*t*BuOK).[284] Unsurprisingly, methanolysis to give **419** was the outcome, Scheme 35. Condensed state experiments with **385** were performed in dilute solutions and at elevated temperatures (> 170 ˚C) for several days with no conversion of **385** observed. Flow

pyrolysis was utilized with a glass–packed reaction tube so as to ensure a high surface area for a reaction center. A solution of **385** in methylene chloride was injected into the argon-filled reaction tube with a steady flow of argon gas. Two receiving flasks in tandem were used to collect material and were cooled in liquid nitrogen. Multiple experiments were performed in this manner and the only material obtained was **385** in ranges of 75 – 85%.

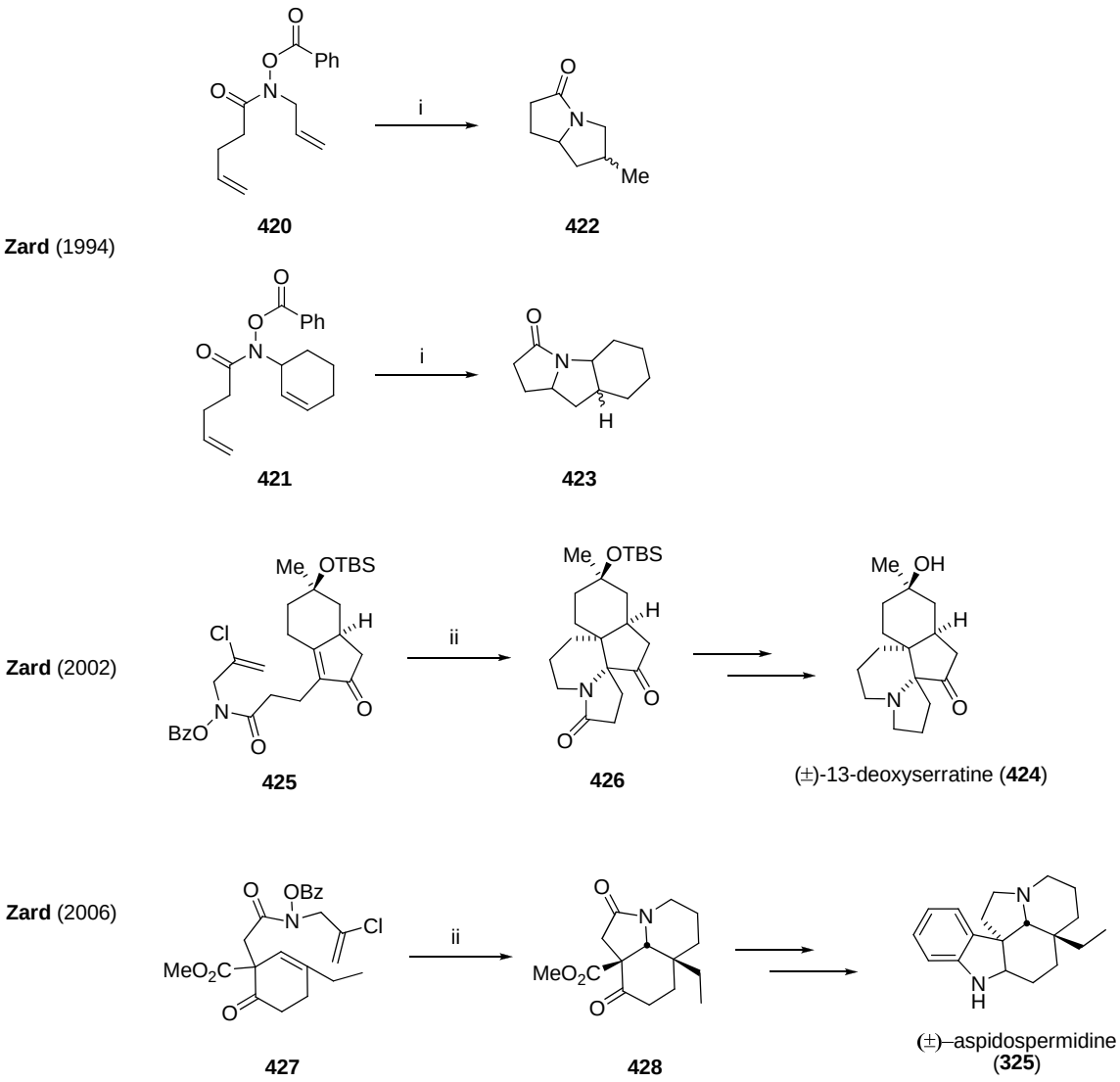


Reagents: (i) *t*BuOK, MeOH, rt (quant); (ii) xylenes (0.05 M), 170 °C → 190 °C, sealed tube; (iii) 340 °C, glass packed reaction tube (85% **385** recovered); (iv) argon blanketed reaction vessel, bunsen burner.

Scheme 35. Solvolysis of hydroxamic acid **385**.

3.5.2 6-*endo*-trig Cyclization *via* Amidyl Radical Approach

Zard has dedicated most of his professional career to the development of radical–based methodology and the synthesis of complex natural products by using radically themed techniques.[8, 267, 285-290] Zard first extended his methodology on the homolysis of the nitrogen – oxygen bond by stannane mediated reactions with *O*-benzoyl hydroxamic derivatives such as **420** and **421** in 1994, Scheme 36.[285] In 1998 Newcomb published the most comprehensive source to date for amidyl radical kinetic information.[291] The rate constants of the reactions involving intramolecular cyclizations of amidyl radicals and the corresponding hydrogen radical transfers have been tabulated. These kinetic considerations were used by Zard in his syntheses of (±)-13-deoxyserratine (**424**)[288] and (±)-aspidospermidine (**325**),[8] Scheme 36. The tandem and regioselective strategy for the 5-exo/6-endo-trig cyclizations included the use of a vinyl chloride and two equivalents of stannane in order to affect the cyclization and subsequent dehalogenation.



Reagents: (i) *n*Bu_3SnH, AIBN (cat), 4 h addition, cyclohexane, reflux (for **422**: 73%; for **423**: 70%); (ii) *n*Bu_3SnH (2 equiv), ACCN, trifluorotoluene (for **424**: 52%; for **325**: 53%).

Scheme 36. Zard 5-exo/6-endo-trig tandem cyclization strategy.

Zard's tandem cyclization strategy for the synthesis of aspidospermidine (**325**) was the inspiration for our amidyl radical-based approach to the vinca alkaloid series ,Figure 60. Our approach to radical precursor **429** is divergent from intermediate carboxylic acid **397** that was used for the thermal approach, Figure 60.

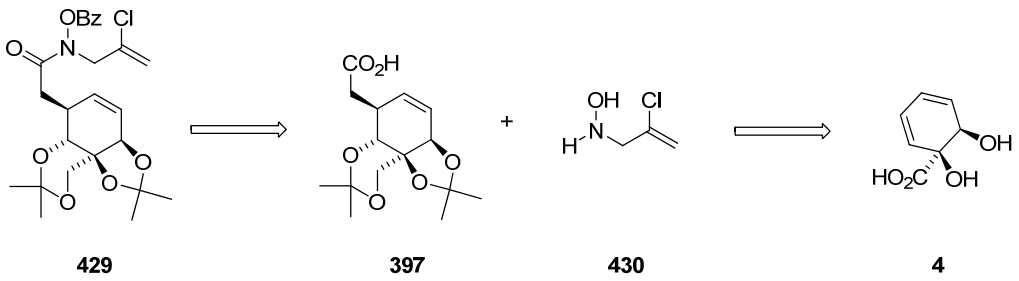
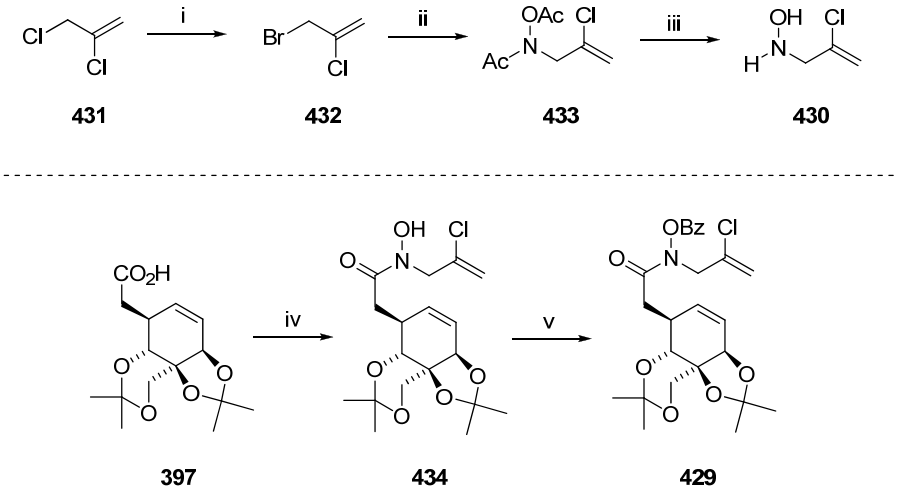


Figure 60. Synthetic analysis for radical precursor **429**.

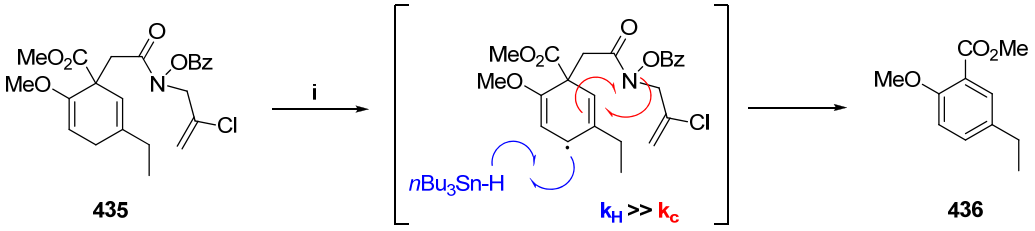
The preparation of *N*-(2-chloroallyl)hydroxylamine **430** was prepared *via* Finkelstein reaction with 2,3-dichloropropene **431** and sodium bromide.[292] 3-Bromo-2-chloropropene **432** underwent substitution with *N*,*O*-diacetyl hydroxylamine **415** to yield **433**. *N*-Acetoxy acetamide **433** was hydrolyzed to give *N*-alkylated hydroxylamine **430** which was subsequently coupled with carboxylic acid **397** to give **434** and then benzoylated to yield **429**, Scheme 38.



Reagents: (i) NaBr, DMF, 90 °C (51%); (ii) AcHNOAc **415**, K_2CO_3, DMF (35%); (iii) HCl (aq, 1 M), reflux (77%); (iv) isobutylchloroformate, Et_3N, THF, 0 °C; then **430** (56%); (v) BzCl, Et_3N, CH_2Cl_2, 0 °C (86%).

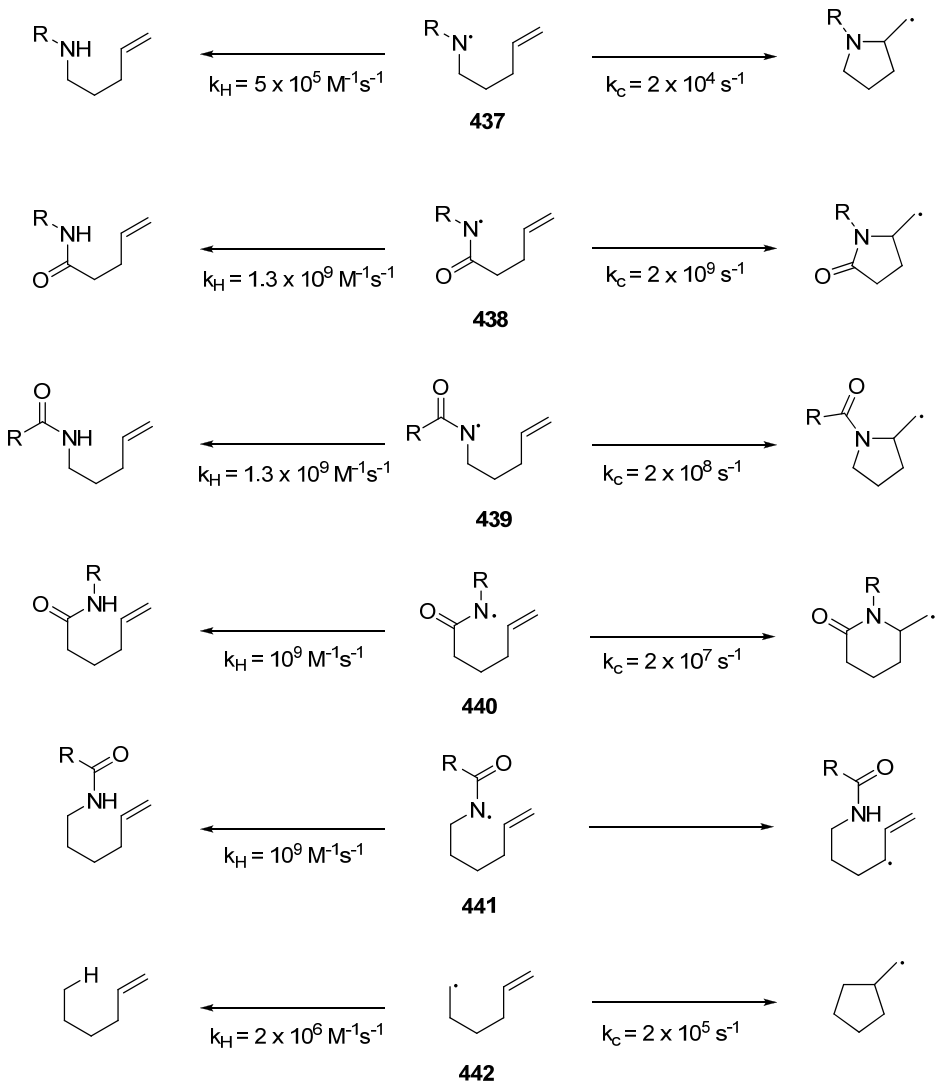
Scheme 37. Synthesis of hydroxyl amine **430** and radical precursor **429**.

A concern with this radical based approach was addressed by Zard confronted in his report on the synthesis of aspidospermidine (**325**), namely the kinetics of allylic hydrogen transfer versus the cyclization *via* nitrogen centered radical.[8] The proton transfer from tin hydride at the allylic carbon of Zard's intermediate **435** proved to outcompete the rate of cyclization and yielded aromatic ester **436**, Scheme 38. Newcomb's empirically determined rate of cyclization versus rate of proton transfer are generalized below, **437 – 442**, Scheme 39.



Reagents: (i) nBu$_3$SnH, ACCN, trifluorotoluene.

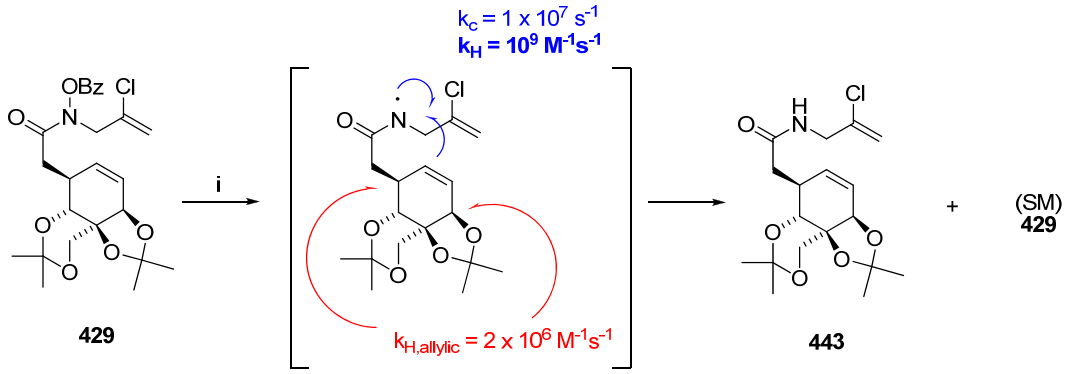
Scheme 38. Hydrogen abstraction from allylic ester **435**.



Scheme 39. Rate constant approximations for cyclization (k_c) *vs*. hydrogen transfer (k_H).[291]

The concern with the kinetic pathway for intermediate **429** is not unfounded for compounds **440** and **441**, Scheme 39, and in view of consideration Zard's outcome for **435**, Scheme 38. The hydrogen transfer rate constant for amidyl radical **440** is 100 times that of the rate constant for the corresponding cyclization. The allylic atom hydrogen transfer is overwhelmingly favored for **441**, as reported by Zard.[290] Unfortunately, the outcome of treating **429** with stannane and radical initiator substantiated reports and the tandem 5-*exo*-trig/6-*endo*-trig cyclization could not be executed as desired, Scheme 40.

The reaction profile was variable for the cyclization attempts of **429**. Special care was taken to degas reagents and reaction mixtures, dilute reaction conditions were used (≥ 0.02 M), and addition times spanned several hours. The only material isolated, other than recovered **429**, was amide **443** as a result of the substantially faster reaction rate of the hydrogen extraction from the reagent (tin hydride) or transfer from one of the two allylic positions where C-centered radical may also extract a hydrogen atom from the reagent.



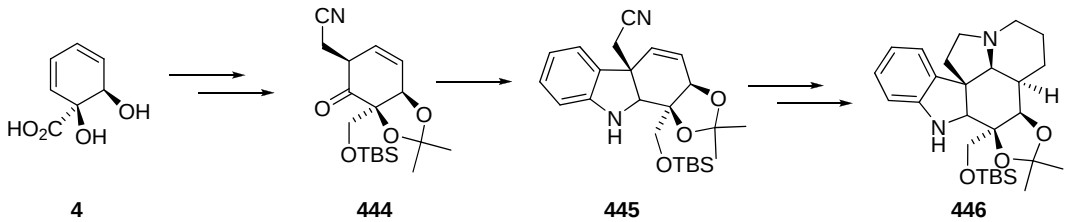
Reagents: (i) nBu$_3$SnH, ACCN, PhCF$_3$, reflux (13 h addition of reagent) (> 80% **429**).

Scheme 40. Amidyl radical generation of **429**.

3.5.3 Miscellaneous Indolization, Claisen Rearrangement, and Diels-Alder Approaches

Construction of Indoline Core

The construction of the indoline core prior to the functionalization of the carbocycle was explored with intermediate **444** in a Fischer indolization to generate **445**, as shown in, Figure 61.

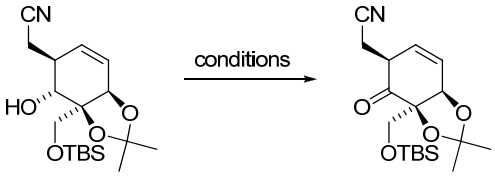


4 444 445 446

Figure 61. Synthetic rationale for indoline **446**.

The synthesis of alcohol **409** was previously demonstrated, Scheme 33, and the oxidation to provide **444** could be executed in good yield (81%) by using Dess-Martin periodinane, Table 14.

Table 14. Oxidation of **409**.

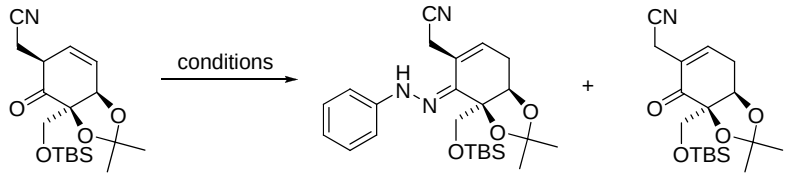


409 **444**

Entry	Conditions	Results
1	IBX (2.0 equiv), EtOAc, rt → 50 °C, sealed tube, overnight – non-aqueous workup	Slow and incomplete conversion to **444**
2	CrO_3/Ac_2O, DCM, rt (titration)	**444** (28%)
3	CrO_3/Ac_2O, DCM, 0 °C (titration)	**444** (54%)
4	IBX (2.0 equiv), EtOAc, 30 °C, 3 days – aqueous workup	Slow conversion to **444**, appearance of byproduct after workup procedure
5	Dess-Martin periodinane (2.5 equiv), DCM	**444** (81%)

When the β,γ–unsaturated ketone **444** was subjected to classical Fischer indolization conditions isomerization occurred to form α,β-unsaturated phenyl hydrazone **447**. Enone **444** was, in fact, sensitive to acidic conditions and upon treatment with acetic acid at room temperature isomerized to **448**. Pyridinium *p*-toluenesulfonate (PPTS) was used as a weak acid catalyst and zinc chloride ($ZnCl_2$) as a Lewis acid without any evidence of indolization, Table 15.

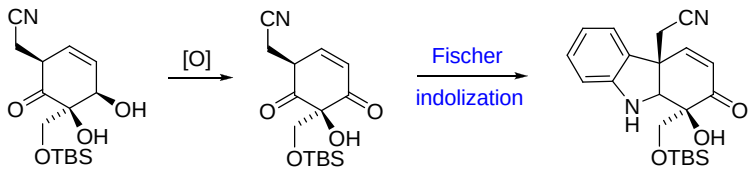
Table 15. Fischer indolization attempts on **444**.



444 **447** **448**

Entry	Conditions	Result
1	Phenylhydrazine, HOAc (glac), 95 °C	**447** (62%) and **448** (17%)
2	Phenylhydrazine, PPTS, toluene, 111 °C	complex mixture
3	Phenylhydrazine, EtOH/H_2O, RT	complex mixture
4	Phenylhydrazine, $ZnCl_2$, MeOH, RT	**448**
5	HOAc (aq), EtOH	**448**

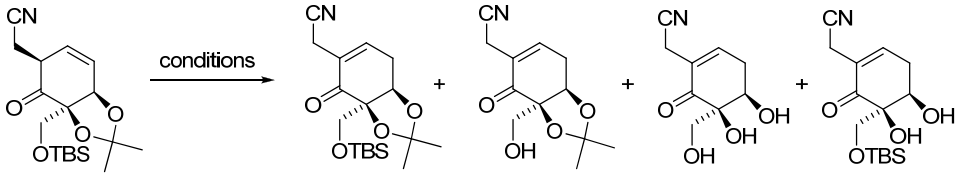
In order to circumvent the isomerization from **444** to **448** and thus construct the indoline skeleton **449**, *via* **450**, Figure 62, the deprotection of **444** was attempted, Table 16. Protecting group incompatibility prevented any selectivity for diol **451** and mixtures of products were obtained that contained the isomerized protolysis products **452** – **454**.



451 450 449

Figure 62. Indolization of ene-dione **450**.

Table 16. Deprotection of **444**.

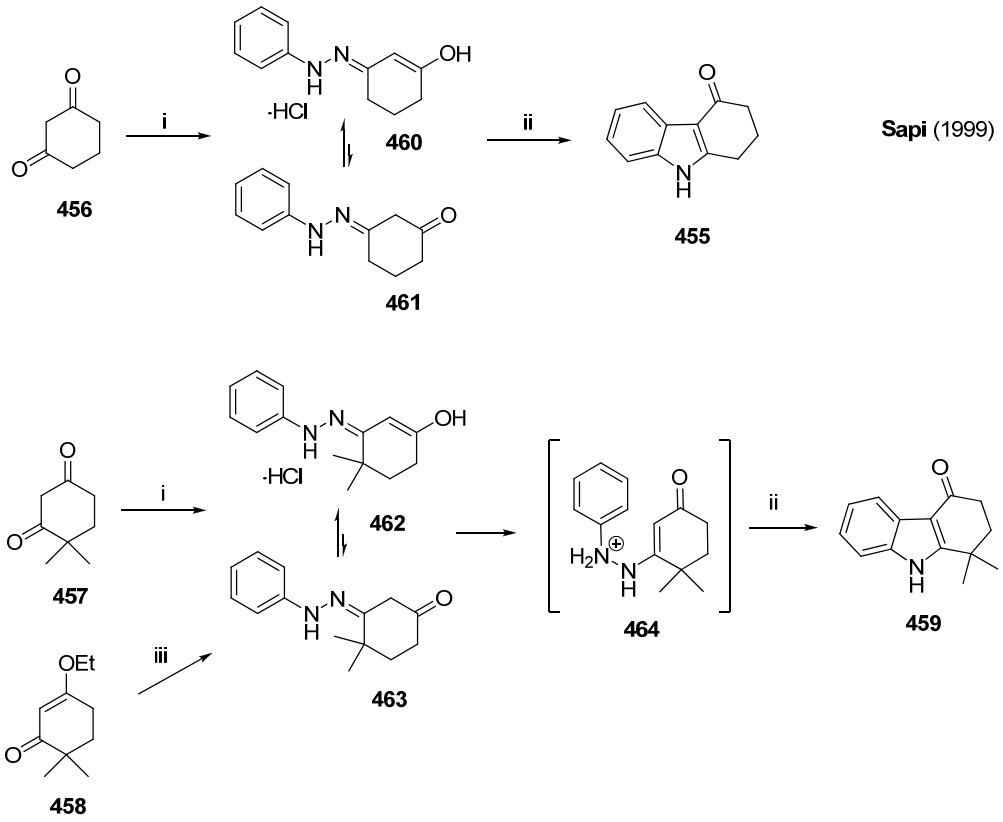


444 448 452 453 454

Entry	Conditions	Result
1	HCl, EtOH, rt	**452**
2	HCl, THF, rt	**448** (50%)
3	TFA, THF/H_2O, 0 °C → rt	complex mixture
4	Amberlyst 15 (H^+), dioxane/H_2O, RT → 55 °C	**452** and **453**
5	HOAc(aq), 55 °C	**452** and **453**
6	Kim's reagent ($FeCl_3 \cdot SiO_2$), $CHCl_3$[293]	**444**, **453** (trace), **454** (trace)
7	$CeCl_3 \cdot 7H_2O$, $(CO_2H)_2$, CH_3CN[294]	complex mixture, **452** (28%)
8	HOAc(aq), ethylene glycol, 50 °C	**453**, degradation
9	$BF_3 \cdot OEt_2$, 1,3-dithiolpropane, CH_2Cl_2, 0 °C[295]	**448** (75%)

Unable to generate intermediate **450** to explore indolizations of ene-1,5-diones, Fischer indole synthesis was pursued with model enones based on the work of Sapi.[296] Sapi prepared indoles of type **455** and **456** from the corresponding 1,3-dione **456**, Scheme 41. Sapi's reaction conditions were applied to both 1,3-dione **457** and to keto-

enol **458** to form indole **459**. The tautomeric phenyl hydrazones (**460** – **463**) provided the electronic "assistance" to generate the required ene-hydrazine **464** crucial for the [3, 3]-sigmatropic rearrangement in forming the indole nucleus.



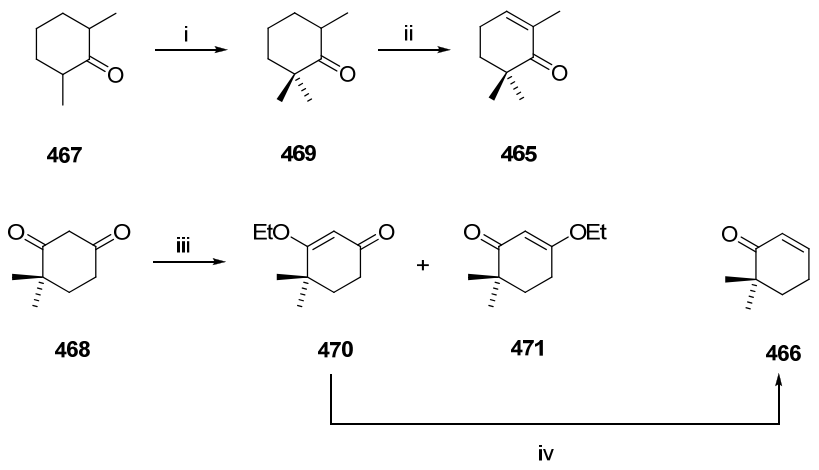
Reagents: (i) $PhNHNH_2$·HCl, HOAc (aq), 40 °C (for **460/461**: 45%; for **462/463**: 52%); (ii) H_2SO_4, 100 °C (for **455**: 32%; for **459**: 26%); (iii) $PhNHNH_2$·HCl, EtOH, reflux (43%).

Scheme 41. Fischer indolization of 1,3-diketones.

Any extension of the reaction conditions demonstrated with 1,3-diones toward the indolization of α,β-unsaturated phenyl hydrazone **447** or enone **448** failed, even when a good Michael donor (triphenyl phosphine, Ph_3P) was provided in an attempt to form a tautomeric species, such as in the case of **460 - 463**.

Model enones **465** and **466** were prepared from **467** and **468**, respectively, along with the trisubstituted cyclohexanone **469**, to be subjected to various indolization

conditions, Scheme 42.[297, 298] The alkylation of diketone **468** generated a mixture of keto enol-ethers **470** and **471** and the minor component **470** was reduced to enone **466**.

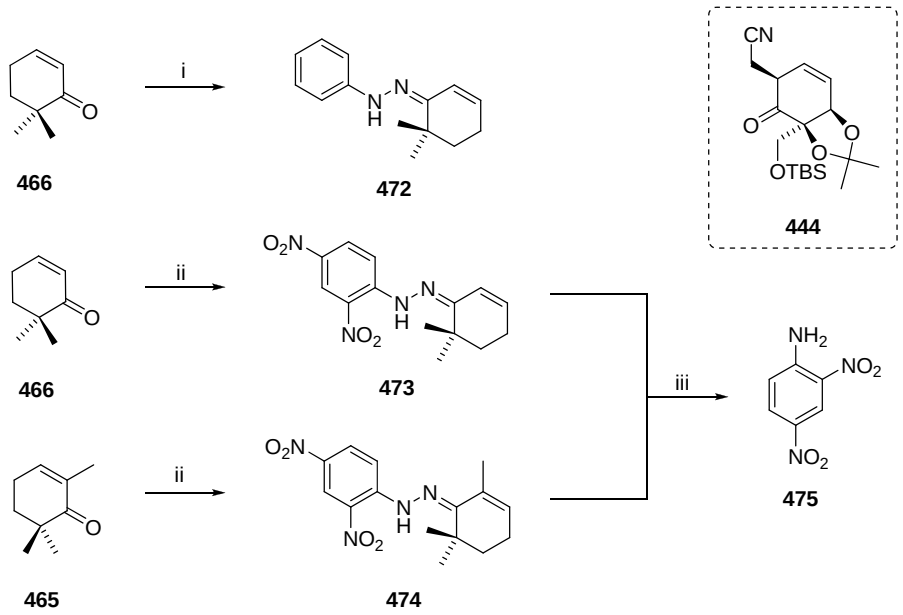


Reagents: (i) lithium diisopropyl amine (LDA), THF, -55 °C; then MeI (83%); (ii) Br_2, pyridine, CH_2Cl_2, reflux (59%); (iii) *p*TsOH, EtOH, PhH, reflux (**470**: 20%; **471**: 72%); (iv) $LiAlH_4$, Et_2O, 0 °C → rt (87%).

Scheme 42. Preparation of model enones **465** and **466**.

Conditions were applied to these model compounds with the hope to apply similar conditions eventually to ketone **444**. Limited success was achieved in finding general conditions for the electronically and sterically similar models, Scheme 43. The treatment of enone **466** with phenylhydrazine hydrochloride provided α,β-unsaturated phenylhydrazone **472** in low yield and as a component of a complex mixture. Alternatively, the formation of the typically more stable 2,4-dinitrophenylhydrazone **473** was facile and high yielding. However, the electronic effects of the nitro groups in **473** and **474** favored a dissociative pathway and only 2,4-dinitro aniline **475** was isolated.[299]

With no success in finding conditions to transform substrate **444**/**447**/**448** into the corresponding indoline compound, the saturated carbocyclic intermediate **476** was prepared, Scheme 44.

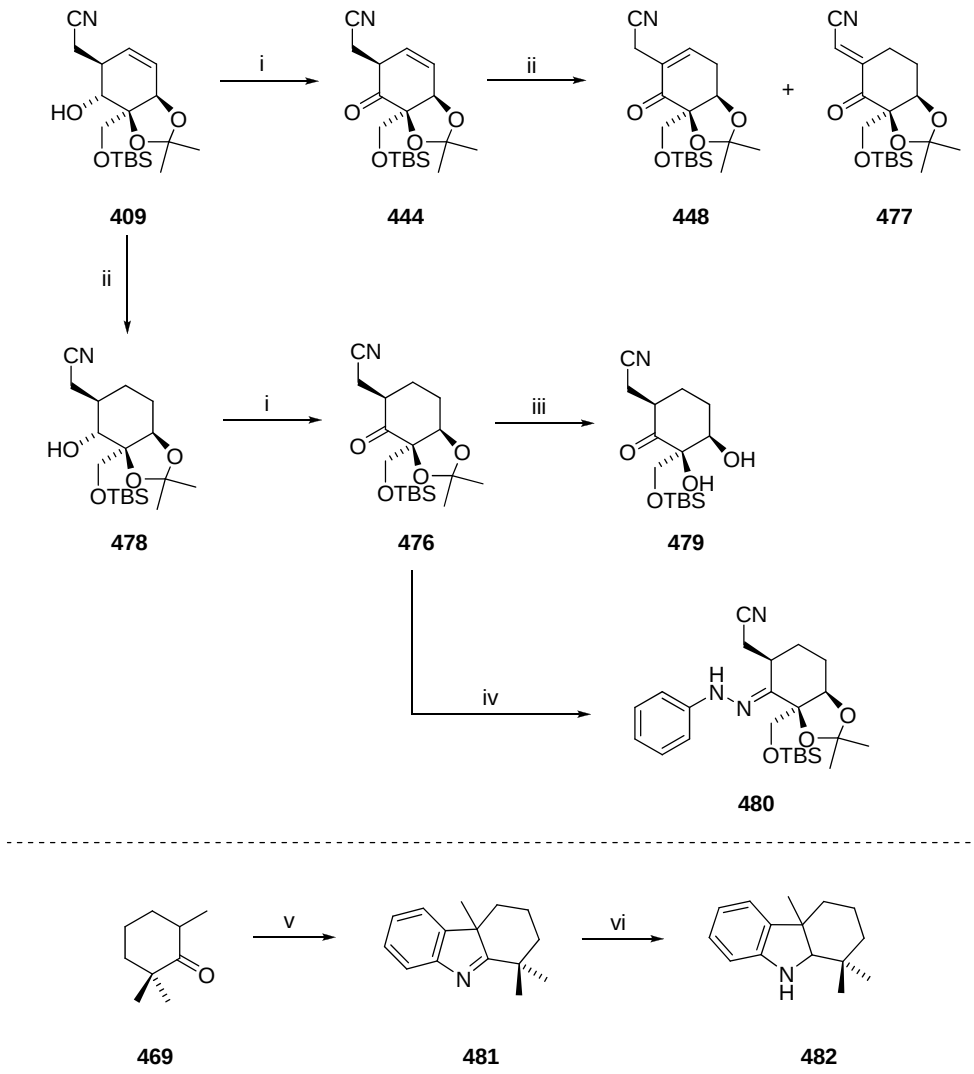


Reagents: (i) $PhNHNH_2$·HCl, EtOH (4%); (ii) 2,4-dinitrophenylhydrazine, HCl, EtOH, reflux (**473**: 89%; **474**: 39%); (iii) HCl (conc) or HOAc, reflux.

Scheme 43. Fischer indolization of enones - model compounds.

When ketone **444** was subjected to hydrogenation conditions (Pd/C) the isomerized products **448** and **477** were formed in a 2:1 ratio, likely because of isomerization tendency to form the more stable, or highly substituted, olefin commonly seen when using Pd/C as a catalyst. No hydrogenated product **476** was obtained even with increased hydrogen pressure and time. To this end, alcohol **409** was hydrogenated under the same conditions and afforded **478** in 90% yield. The saturated alcohol **478** was oxidized with Dess-Martin periodinane to provide **476**. Ketone **475** was subjected to acid catalyzed indolization conditions and afforded protolysis product **479** and several polar impurities. Phenylhydrazone **480** was formed when **476** was treated with phenylhydrazine hydrochloride in pyridine but conversion to **480** was low and no indoline product was afforded, despite the successful cyclization of a model compound **469** to imine **481** and subsequently reducing to indoline **482**.[300] The bulkiness of the TBS-silyl either in **476**

was suspected to be a disabling factor in terms of adequate orbital overlap of the phenyl group of the phenylhydrazone **480** to undergo the sigmatropic rearrangement. The hypothesis was never empirically substantiated.



Reagents: (i) Dess-Martin periodinane, CH_2Cl_2 (for **444**: 81%; for **476**: 70%); (ii) Pd/C, H_2, EtOH (for **448/477**: 40%, 1:0.5 ratio; for **478**: 70%); (iii) $PhNHNH_2$·HCl, EtOH, reflux (25%); (iv) $PhNHNH_2$·HCl, pyridine, reflux (15% **480**, 63% recovered **476**); (v) $PhNHNH_2$, HOAc (glac) (80%); (vi) $NaBH_3CN$, HOAc (glac) (quant).

Scheme 44. Indolization attempts with ketone **476**.

Claisen Approach

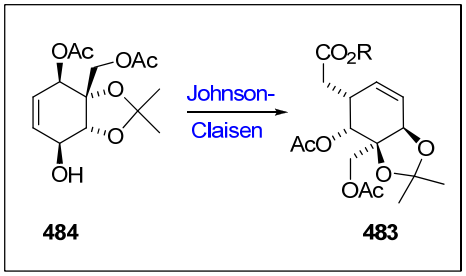
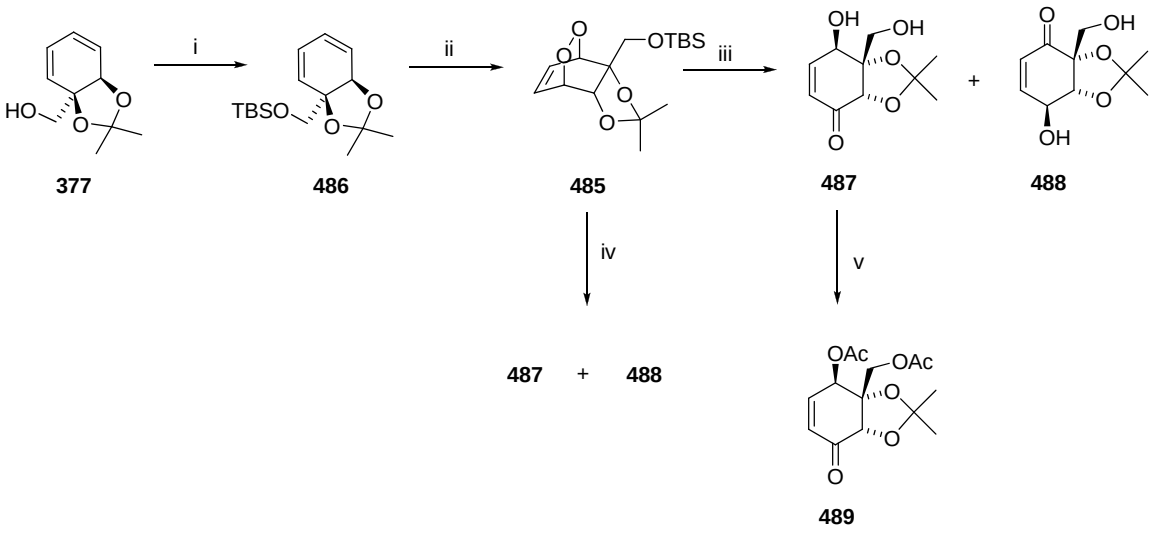


Figure 63. Johnson-Claisen intermediate.

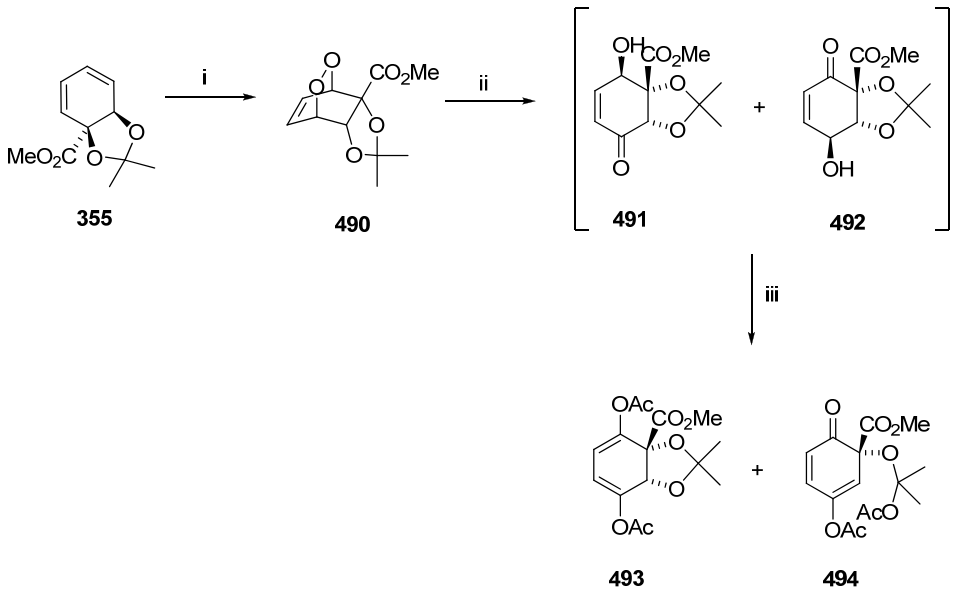
An approach based on Johnson – Claisen rearrangement was envisioned that would provide intermediate **483**, diastereomeric at the allylic position, from an allylic alcohol such as **484**, Figure 63. The route incorporated the Kornblum – DeLaMare rearrangement[301] of endoperoxide **485**, recently reported by Lewis, Scheme 45.[302] Alcohol **377** was protected as the silyl ether **486** and then subjected to photolysis in the presence of sensitizer (tetraphenylporphyrin, TPP) to generate endoperoxide **485**. The regioselectivity reported by Lewis indicated that the intramolecularly initiated Kornblum–DeLaMare rearrangement *via* primary alkoxide was entirely selective for **487**. When endoperoxide **485** was treated with tetrabutylammonium floride (TBAF) a ratio of 1:1.2 of **487**/**488** was observed in 78% yield. Dilution factors and reaction conditions could not affect a better distribution of **487**/**488**. The mixture of enones **487**/**488** were labile and only small amounts of acetate **489** were obtained. The poor regioselectivity of the Kornblum–DeLaMare rearrangement deemed this route a dead end.



Reagents: (i) TBSOTf, Et_3N, CH_2Cl_2 (96%); (ii) O_2, TPP, CCl_4, 5 °C (70%); (iii) TBAF, THF, -20 °C → rt (**487**: 35%; **488**: 43%); (iv) $(iPr)_2EtNH$, CH_2Cl_2 (**487**: 29%; **488**: 18%); (v) Ac_2O, Et_3N, DMAP, CH_2Cl_2 (12%).

Scheme 45. Johnson–Claisen approach.

The Kornblum–DeLaMare rearrangement of methyl ester **355** gave equally complex mixtures and isolation of useful intermediates was futile, Scheme 46. Degradation of endoperoxide **490** with Hunig's base yielded a mixture of enones **491**/**492** (1.4:1) and upon acylation isomerization products **493** and **494** were isolated in low yields.

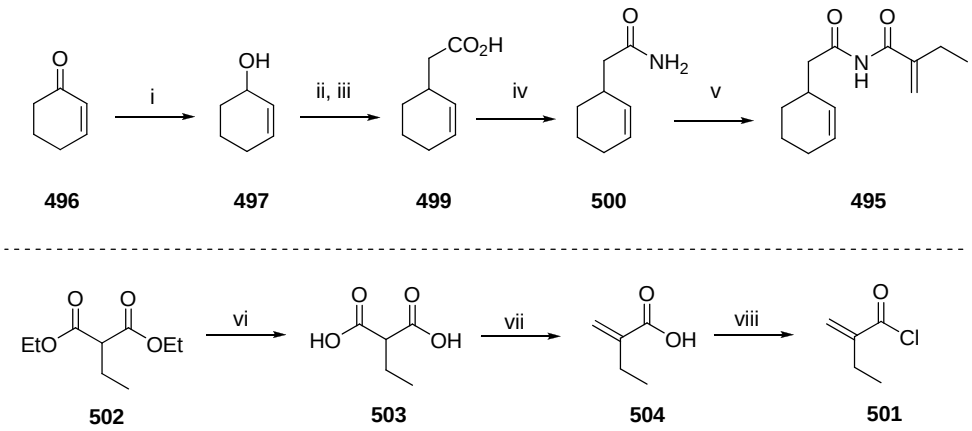


Reagents: (i) O_2, TPP, CCl_4; (ii) $(iPr)_2EtNH$, $CDCl_3$, rt (**491**/**492** 1.4:1 ratio); (iii) Ac_2O, Et_3N, DMAP, CH_2Cl_2 (**493**: 6%; **494**: 4%)

Scheme 46. Kornblum–DeLaMare rearrangement of **355**.

Imidate Cycloaddition Model

A model imidate system was prepared in order to test the feasibility of a cycloaddition approach from **495**, Scheme 47. Cyclohexenone **496** was reduced under Luche conditions to afford allylic alcohol **497**. Johnson–Claisen rearrangement was performed on **497** and the resulting ester **498** subjected to saponification to yield carboxylic acid **499**.[303] The acid chloride derived from **499** was prepared and treated with liquid ammonia to provide primary amide **500**. Treatment of amide **500** with *n*-butyl lithium and quenching the resulting anion with acryloyl chloride **501**, prepared in three steps from diethyl 2-ethylmalonate **502**,[304] provided imide **495**.

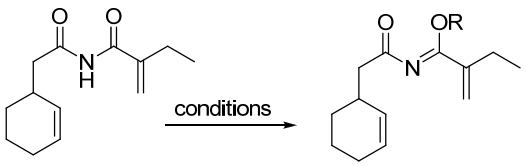


Reagents: (i) $CeCl_3{\cdot}_7H_2O$, $NaBH_4$, MeOH, 0 °C (51%); (ii) triethyl orthoacetate, propionic acid, 140 °C (60%); (iii) NaOH, MeOH (93%); (iv) $(COCl)_2$, PhMe, rt; then NH_3 (l), -70 °C (86% from **499**); (v) *n*BuLi, THF, -65 °C; then **501** (30%); (vi) KOH (aq), reflux (53%); (vii) $Et_2NH{\cdot}HCl$, HCHO (aq), reflux (58%); (viii) $(COCl)_2$, Et_3N, Et_2O, 0 °C.

Scheme 47. Synthesis of model imide **495**.

A series of alkylations were attempted on the stabilized functional group without the successful formation of an imidate, Table 17.

Table 17. *O*-alkylation attempts on imidate **495**.

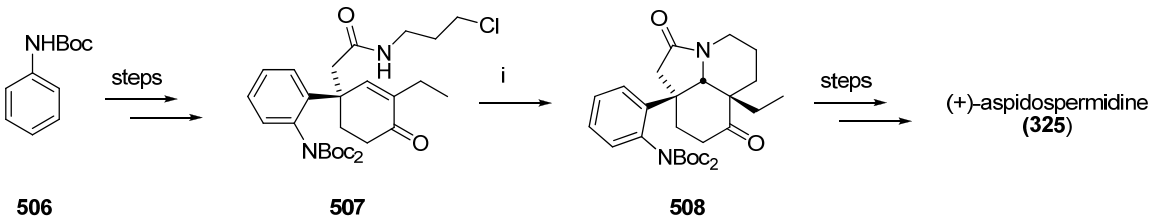


495 **505**

Entry	Conditions	Result
1	*n*BuLi, TMSCl, THF, -65 °C → RT	**495**
2	NaH, TMSCl, THF, 0 °C→ RT	**495**
3	Meerwein reagent (trimethyloxonium tetrafluoroborate), DCM, 0 °C → RT	**495**
4	Bis(TMS)trifluoroacetamide, MeCN, RT → 110 °C	**495**
5	MeI, NaH, THF, 0 °C	**495** + trace polar material

3.5.4 Conjugate Addition – Alkylation Approach

In 2002 Marino and co-workers reported on the total synthesis of (+)-aspidospermidine (**325**) in 18 steps from Boc-protected aniline **506**.[9] A key step in his synthesis was a tandem conjugate addition–enolate alkylation of enone **507** to form the tricyclic intermediate **508**, Scheme 48.



Reagents: (i) NaH, DMF, 0 °C (86%).

Scheme 48. Marino's (+)-aspidospermidine synthesis – formation of tricycle **508**.

The preparation of an intermediate enone, such as **387**, Figure 64, was pursued in order to implement a tandem process similar to that Marino *et al*. published. To initiate the approach toward enone **387** the secondary alcohol of **409** needed to be masked. The benzyl (Bn) group provided compatibility with the other protecting groups and would allow for maneuvering during the course of re-functionalizing the carbocycle. However, the protection did not turn out to be trivial and several products were isolated, including unreacted **409** and **509 – 511**, Scheme 49. The addition of tetrabutylammonium iodide to the reaction provided the kinetic advantage, because of the increased reactivity of benzyl iodide, to suppress formation of side products.

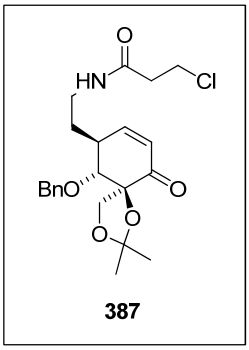
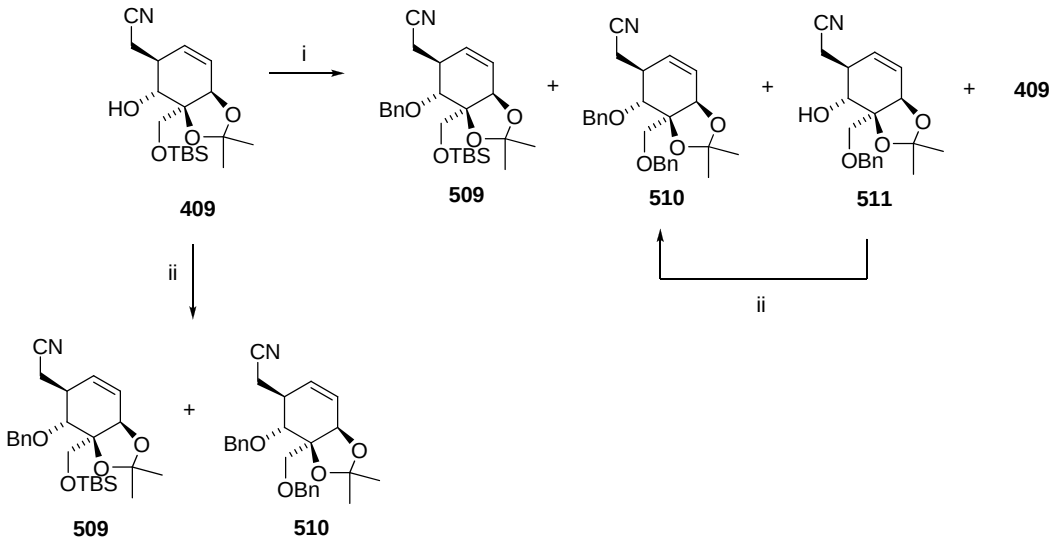


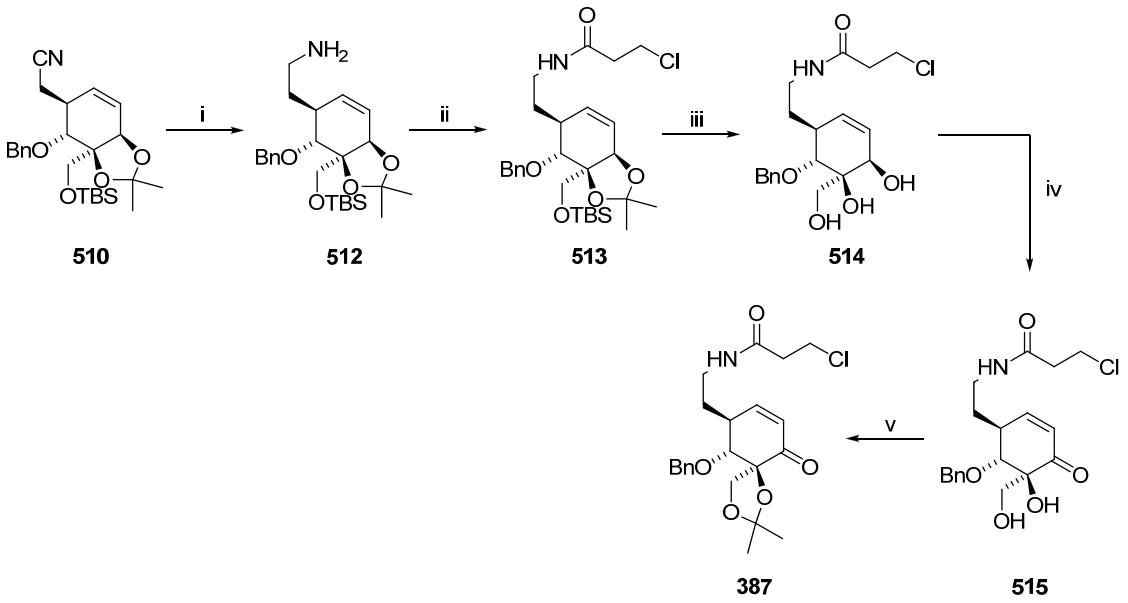
Figure 64. Enone **387**.



Reagents: (i) BnCl, NaH, THF, reflux (**409**: 13%; **509**: 2%; **510**: 5%; **511**: 60; (ii) BnCl, Bu_4NI, NaH, THF, reflux (**511**: 70%; **510**: 10%; for **511** → **510**: 79%).

Scheme 49. Benzylation of **409**.

The benzyl nitrile derivative **509** was treated with lithium aluminum hydride in diethyl ether to afford amine **512**, Scheme 50. The reduction was followed with acylation and subsequent deprotection to triol **514**. The allylic alcohol was selectively oxidized with manganese dioxide (MnO_2, 100% conversion) to **515** as evidenced by TLC analysis. However, yields in the range of 35 – 40% were typical, presumably because of adsorption of the polar substrate to the surface of the reagent. Isopropylidine **387** was prepared under standard conditions by treatment with dimethoxypropane and *p*-toluenesulfonic acid.



Reagents: (i) $LiAlH_4$, Et_2O, rt (94%); (ii) 3-chloropropanoyl chloride, CH_2Cl_2, $NaHCO_3$ (aq) (quant); HCl (conc), MeOH (85%); (iv) MnO_2, acetone (40%; 100% conversion); (v) 2,2-DMP, *p*TsOH, acetone (83%).

Scheme 50. Synthesis of enone **387**.

Having already prepared one of the precursors for the cyclization, namely **387**, an alternative enone amide **388** was also prepared, Figure 65. The preparation began from the base hydrolysis of benzyl silyl ether **510**. Hydrolysis stopped at amide **516** with concomitant removal of the TBS-group, Scheme 51. The carboxylic acid could not be prepared in any appreciable amounts using these conditions. Acylation of the primary alcohol in **516** with acetic anhydride gave **517**, which upon *N*-alkylation with 1-bromo-3-chloropropane gave alcohol **516**.

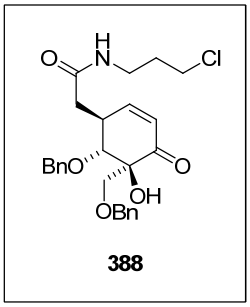
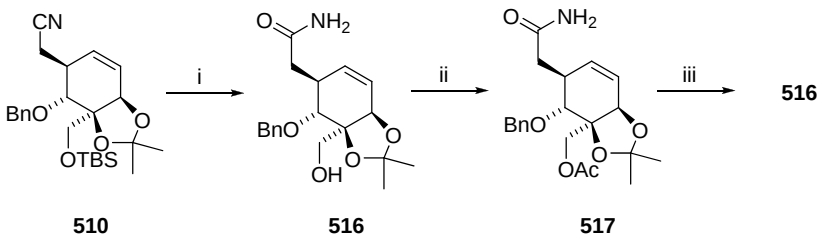


Figure 65. Enone **388**.

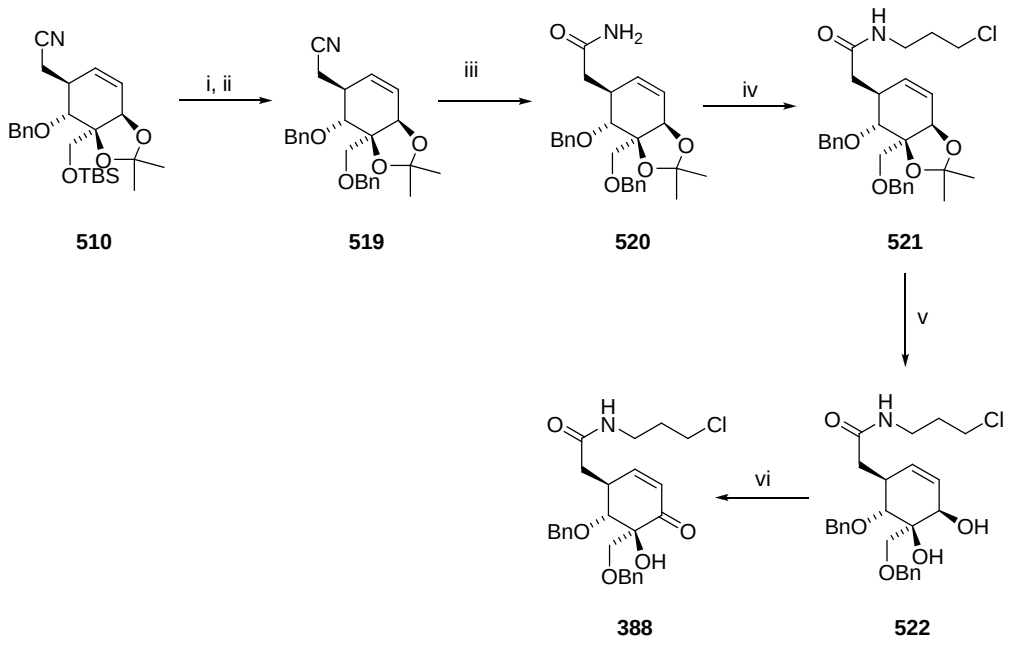


510 **516** **517**

Reagents: (i) NaOH (aq), MeOH, reflux (43%); (ii) Ac_2O, DMAP, Et_3N, CH_2Cl_2 (72%); (iii) NaH, 1-bromo-3-chloropropane, THF (quant).

Scheme 51. Synthesis of amide **519**.

The silyl ether in **510** was removed with fluoride to give **518**, which was in turn alkylated with benzyl chloride to give **519**, Scheme 52. Hydrolysis of **519** gave amide **520** which was alkylated with 1-bromo-3-chloropropane to give **521**. Several hydrolytic conditions were used in order to attempt the removal of the acetonide in **521**. Hydrochloric acid in methanol led to the transesterification of the amide, aqueous acetic acid did not provide **521**, and hydrochloric acid in isopropanol gave a 1:1 ratio of **521**/**522**. Iron(III) chloride adsorbed onto silica gel gave the best results but with large variation in yield (30% - quantitative), regardless of the quality of the reagent. The selective oxidation of **522** with manganese dioxide gave the cyclization precursor **388** in moderate yield.



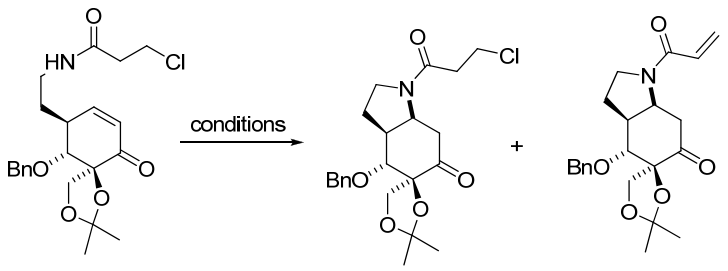
510 **519** **520** **521** **388** **522**

Reagents: (i) TBAF, THF (quant); (ii) BnCl, Bu_4NI, NaH, THF, reflux (70%); (iii) NaOH (aq), MeOH, reflux (83%); (iv) 1-bromo-3-chloropropane, NaH, DMF, rt (56%); (v) $FeCl_3$/silica gel, $CHCl_3$, rt (30% - quant); (vi) MnO_2, $CHCl_3$, rt (**522**: 28%; **388**: 56%)

Scheme 52. Synthesis of precursor **388**.

With cyclization precursors **387** and **388** in hand, the effort toward their cyclization was made. The initial intramolecular Michael addition of **387** to **523**/**524** was facile and could be induced under several conditions, Table 18. The acrylamide **524**, however, was resilient to further enolate addition. The treatment of **524** with Meerwein's reagent (triethyl and trimethyloxonium tetrafluoroborate) yielded material indistinguishable from a carbocyclic skeleton.[305] A report recently published by Qiu and Jin,[306] Table 18 (Entry 9), describes the Michael addition of an acrylamide to and α, β-unsaturated aldehyde followed by the subsequent enolate addition to the acrylamide, a tandem Michael addition. When Qiu and Jin's conditions were applied to enone **387** only the first Michael addition to the enone was successful and the second conjugate addition to the acrylamide failed and yielded **524**.

Table 18. Cyclization conditions for enone **387**.

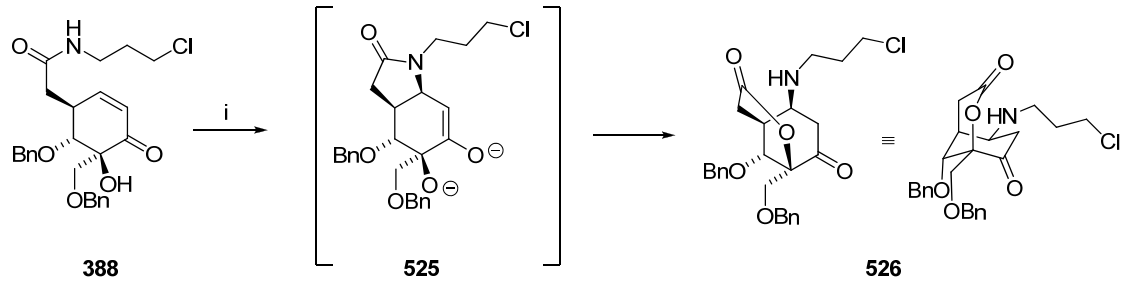


387 **523** **524**

Entry	Conditions	Result
1	NaH (1.0 equiv), DMF, 5 ˚C → rt	decomposition
2	NaH (1.0 equiv), THF, 5 ˚C, 1.5 h	**524** (quant)
3	NaH (1.0 equiv), THF, 5 ˚C, 1 h	**523** (62%) + **524** (18%)
4	PhMe (0.004 M), 180 ˚C, 4 days	no conversion, **387**
5	PPTS, PhMe, 50 ˚C	**523** + **524** (< 2%)
6	DBU (2.0 equiv), THF, rt	**524**
7	*n*BuLi, THF, -80 ˚C → -20 ˚C	**523** + **524** (trace)
8	*n*BuLi, (-)-sparteine, THF, -80 ˚C → -20 ˚C	**523**
9	K_2CO_3, MeCN, 80 ˚C, 8 h	**524**

To prevent the tendency of the halide to eliminate and form a conjugated species, such as acrylamide **524** (from **387**), enone **388** was prepared with the carbonyl functional group placed on the other side of the nitrogen.

A solution of enone **388** was treated with sodium hydride (2 equivalents) at 0 ˚C. These conditions were previously used with enone **387** and afforded Michael addition product **523** in good yield. However, no conversion of enone **388** was observed under this reaction conditions and starting material was recovered. When **388** was treated with pyridinium *p*-toluenesulfonate in methylene chloride no reaction occurred and starting material was also recovered. However, upon treatment of **388** with sodium hydride (2 equivalents) in DMF cyclization occurred, initially forming lactam **525**, and was followed by transesterification (lactonization) of the lactam with the tertiary alkoxide in **525** to generate **526**, Scheme 53. The proximity of the tertiary alkoxide in **525** to the lactam moiety and the conformational stability of product **526** (bicyclo[3.3.1]nonane-like skeleton) combine to overcome the thermodynamic stability of amide **525** and result in the trans lactonization to afford **526**. Further effort to manipulate the chemoselectivity of intermediate **388** toward intramolecular cyclizations, such as masking the tertiary alcohol or heating mixtures that contained **526**, would be worthwhile.



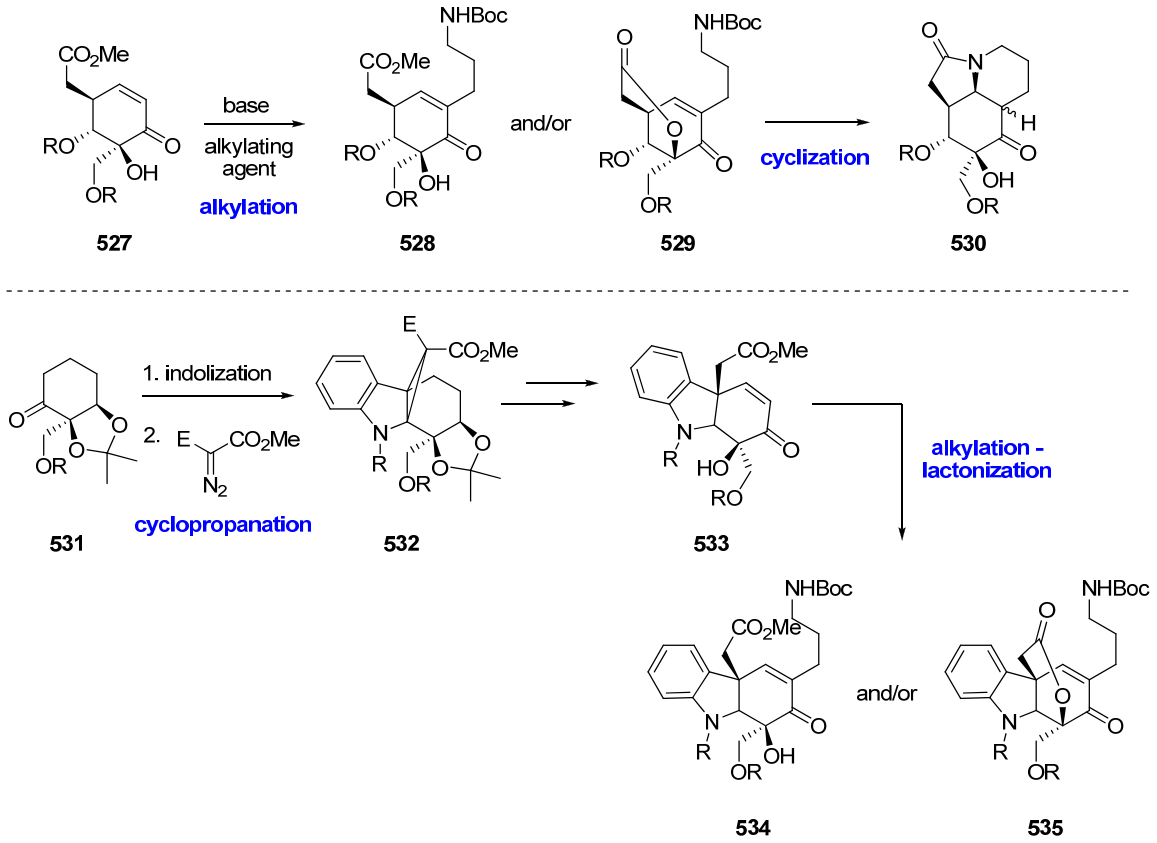
Reagents: (i) NaH (2.0 equiv), DMF, 0 °C → rt.

Scheme 53. Cyclization of enone **388**.

4.0 Conclusions and Future Work

Microbial metabolites derived from whole-cell biotransformations have proven to be useful enantiomerically enriched raw materials. Chemoenzymatic syntheses can provide a significant advantage over traditional chemical methods. It is hoped that the body of the work presented here helps to emphasize the versatility of metabolites such as (–)-3,5-cyclohexadiene-1,2-diol-1-carboxylic acid (**4**) that was generated from benzoic acid (**3**) *via Ralstonia eutrophus* B9 cell line. In doing so the chemoenzymatic synthesis of the natural product (–)-idesolide (**5**) was prepared in four chemical steps, the polyhydroxylate pyrrolidine **6** was synthesized in 13 chemical steps, and several approaches toward the total synthesis of vindoline (**7**) have been presented.

Future effort for the synthesis of vinca alkaloids could utilize intermediates as shown in Scheme 54. The propensity of the tertiary alcohol in enone **528** to undergo transesterification to **529**, as demonstrated with **388** → **526**, could lead to a thermodynamically favored cyclization that would yield a tricyclic amide such as **530**. An alternative synthetic route would first build up the indoline skeleton from ketone **531** by means of Fischer indolization followed by cyclopropanation to afford **532**. Degradation of the cyclopropane ring at C-2 (indole numbering) of indoline **532** would unmask the two-carbon electrophilic handle in **533** and allow another opportunity for an alkylation-lactonization-cyclization sequence with intermediates **534/535**. A brief outline of this future work is shown below.



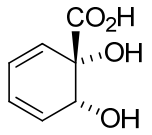
Scheme 54. Future work for synthesis of vinca alkaloids.

5.0 Experimental Section

5.1 General Experimental Details

All non-hydrolytic reactions were carried out under an argon atmosphere. Glassware used for moisture-sensitive reactions was flame-dried under vacuum and subsequently purged with argon. THF was distilled from potassium/benzophenone. Methylene chloride and acetonitrile were distilled from calcium hydride. Flash column chromatography was performed using Silicycle SiliaFlash P60 silica gel (40–66 μm).. Analytical thin-layer chromatography was performed using silica gel 60-F_{254} plates. Melting points were measured on a Thomas-Hoover melting point apparatus and are reported uncorrected. IR spectra were obtained on a Perkin-Elmer FT-IR 1600 Series Spectrum One instrument and were recorded as neat samples. ^{1}H and ^{13}C NMR spectra were obtained on either a 300 MHz (75 MHz) or 600 MHz (150 MHz) Bruker spectrometer. Data are reported as (s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, m = multiplet, br = broad; coupling constants(s) in Hz, integration). Specific rotation measurements are given in deg cm^3 g^{-1} dm^{-1} and were recorded on a Perkin-Elmer 341 Polarimeter. Ultraviolet spectroscopy was performed using a Perkin-Elmer 8452 A diode array spectrophotometer. Large-scale fermentation was performed in a 15-L B. Braun Biostat C-15 fermenter. Combustion analyses were performed by Atlantic Microlabs, Norcross, Georgia, USA.

General procedure for the biotransformation of benzoic acid (3)

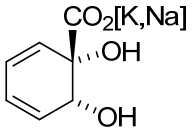


4

(*1S,2R*)-1,2-dihydroxycyclohexa-3,5-diene-1-carboxylic acid (**4**).

The whole-cell biotransformation of benzoic acid was performed based on a modified procedure established by Mihovilovic and co-workers.[69]**Error! Bookmark not defined.** LB(II) medium (100 mL) was inoculated with a single colony of *Ralstonia eutrophus* B9 that was grown on LB(II) agar plates at 30 ˚C for two days. The inoculated medium was incubated at 30 ˚C on an orbital shaker at 185 RPM until OD_{600} = 4.8 (1:10 dilution; ~ 24 h). At this point the cellular suspension (80 mL) was used as a preculture and added to a 15 L Sartorius Biostat C bioreactor that contained HMB medium (8.4 L) at pH = 7.4, aerated with sterile air at 3 L/min, agitation speed of 300 RPM, and D-fructose (50 mL of a 1.5 m aq. solution) concentration of 0.009 m. The culture was grown until an OD_{600} = 2.8 (1:10 dilution) was achieved (~ 20 h) and then induced with sodium benzoate (12 mL of a 1.5 m aq. solution; 18 mmol) and D-fructose (53 mL of a 1.5 m aq. solution; 80 mmol). After 6 h consumption of benzoate was observed by UV analysis (265 nm) and a repetitive feeding program was initiated at which 15 min feeding of an aq. solution of sodium benzoate (22 mL, 1.5 m; 1.5 mL/min feed rate) and aq. D-fructose (22 mL, 1.5 m; 1.5 mL/min feed rate) was performed every 3 hours over the course of 4 days; a total of approximately 170 g of sodium benzoate was fed. After the feeding regime was completed the broth was drained and separated from cell matter by centrifugation at 5 ˚C

(10,000 RPM). The dark brown ferment broth was concentrated *in vacuo* at 35 ˚C to dryness to obtain several lots of *ipso* diene diol carboxylate as the mixed potassium/sodium salts, contaminated with other inorganic salts. NMR spectroscopy assay was used to establish a weight-weight percentage of the crude material with an internal standard (potassium benzoate). A total mass of 261.5 g of crude material was obtained and corresponded to 177 g of the salt of *ipso* diol **337** that could be stored at room temperature without any observable degradation (several months).



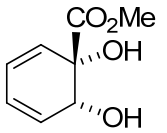
337

337: ^{1}H NMR (600 MHz, D_2O) δ 6.03 (dd, *J* = 9.5, 5.2 Hz, 1H), 5.93 – 5.81 (m, 1H), 5.71 – 5.64 (m, 2H), 4.77 (s, 1H).

The free acid **4** was obtained by the acidification of a cold aqueous solution of the salts with 6 M HCl and extraction with EtOAc. Compound **4** spectral and physical data match those reported in the literature.[66, 69]

4: mp = 94 – 96 (CH_2Cl_2); $[\alpha]_D^{20}$ = 64.9 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, D_2O) δ 6.12 (dd, *J* = 9.3, 5.1 Hz, 1H), 6.05 – 5.90 (m, 1H), 5.77 (d, *J* = 9.6 Hz, 2H), 4.86 (s, 1H).[66]

5.2 Detailed Experimental Procedures

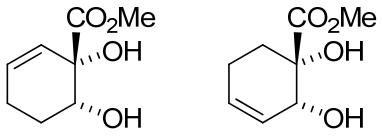


340

(*1S,2R*)-methyl-1,6-dihydroxycylohexa-2,4,-dienecarboxylate (340). To a pre-chilled solution of *ipso* diene diol carboxylic acid **4** (4.0 g, 25.6 mmol) in THF (distilled, 32 mL, 0.8 M) at ~5 °C (ice-bath) was added a preformed solution of diazomethane (excess) in Et_2O dropwise over 30 minutes. The addition of the ethereal solution of diazomethane was continued until consumption of starting material was observed by TLC analysis (1:1 hexanes/EtOAc, CAM). The reaction mixture turned from a slight yellow to a full yellow color over the course of diazomethane addition. The reaction mixture was removed from the ice-bath and allowed to warm to room temperature with stirring. After warming to ambient temperature the reaction mixture was concentrated *in vacuo* to provide orange oil (quantitative by mass). The crude material was chromatographed on silica gel (1:1 hexanes/EtOAc) to yield 3.39 g (78% yield) of diene *ipso* diol methylester **340** as white needle-like crystals after crystallization from Et_2O.

340: R_f = 0.23 (1:1 hexane/EtOAc); mp = 59 – 60 °C (Et_2O); $[\alpha]_D^{20}$ = -96.7 (*c* 1.0, $CHCl_3$); FT-IR (film) ν 3412, 1736, 1641, 1564, 1439, 1407, 1265, 1085, 1044, 945, 854, 816 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 6.17 (q, *J* = 5.1 Hz, 1), 5.98 (d, *J* = 1.3, 9.5 Hz, 1), 5.85 (td, *J* = 1.0, 9.7, 1), 4.87 (s, 1), 3.90 (s, 1), 2.89 (s, 2); ^{13}C ($CDCl_3$, 75 MHz) δ 175.6, 132.0, 126.8, 124.7, 122.7, 73.9, 70.9, 53.7; MS (EI+) *m/z* 170; HRMS cald. for

$C_8H_{10}O_4$: 170.05852; Found: 170.05791. Anal calcd.: C 56.47, H 5.92, O 37.61. Found: C 56.44, H 5.87.



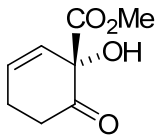
129 **343**

(*1S,6R*)-Methyl 1,6-dihydroxycyclohex-2-enecarboxylate (129).

To a stirred, chilled (ice-bath) solution of ester diol **340** (399 mg, 2.34 mmol) in anhydrous MeOH (5.8 mL) was added potassium azodicarboxylate (PAD) (1.35 g, 7.03 mmol) in portions over 5 minutes. This yellow slurry was allowed to stir at slightly lower temperature (~ 5 °C) for 15 minutes prior to a portionwise addition of a solution of acetic acid (glacial, 11.7 mmol) in MeOH (2 mL) over ~1.5 hours. The rate of addition of HOAc/MeOH was dictated by the rate of N_2(g) evolution (bubbler) observed from the reaction mixture. The reaction was monitored by evolution of nitrogen gas and by TLC (2:1 EtOAc/hexanes) – Note: **129** co-elutes with starting material **340** and can be differentiated by the fact that only **340** is UV active. After the addition was complete the reaction mixture was allowed to stir at ~5 °C for additional 20 minutes prior to slowly warming to room temperature. Once at ambient temperature the reaction mixture was concentrated to dryness and reconstituted in EtOAc (15 mL) to produce a white slurry. The slurry was filtered and the filtrate concentrated to provide a colorless oil (576 mg). The crude oil was chromatographed on silica gel (3:1 → 1:1 hexanes/EtOAc) to yield 155 mg (40%) single isomer **129** as a white crystalline solid; 81% overall yield, combined isomers **129** and **343**.

129: R_f = 0.50 (2:1 EtOAc/hexanes); mp = 68 – 71 °C (EtOAc); $[\alpha]_D^{20}$ = -36.1 (*c* 1.0, $CHCl_3$); FT-IR (film) ν 3441, 2953, 1735, 1436, 1262, 772 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.68 - 1.85 (m, 1 H) 1.86 - 1.97 (m, 1 H) 2.21 - 2.32 (m, 2 H) 3.86 (s, 3 H) 4.04 (dd, *J*=11.7, 3.8 Hz, 1 H) 5.62 (dt, *J*=9.8, 2.1 Hz, 1 H) 6.05 (ddd, *J*=9.7, 4.2, 3.0 Hz, 1 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 175.8, 132.9, 125.4, 73.8, 70.9, 53.4, 26.5, 24.9; MS (EI+) *m/z* 172; HRMS calcd. for $C_8H_{12}O_4$: 172.07, Found: 172.07388. Anal calcd.: C 55.81, H 7.02, O 37.17. Found: C 55.68, H 7.15.

343: light brown oil. R_f = 0.39 (2:1 EtOAc/hexanes); $[\alpha]_D^{20}$ = -21.9 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz) δ 5.85 (md, *J*=1.5, 8.6 Hz, 1 H), 5.58 (qd, *J*=2.0, 10.2 Hz, 1 H), 4.50 (m, 1 H), 2.32 (m, 1 H), 2.08 (m, 1 H), 2.05 – 1.65 (m, 4 H), 1.50 – 1.35 (m, 1 H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 176.3, 129.0, 127.9, 74.5, 72.3, 69.0, 53.2, 34.4, 30.3, 24.1, 21.2, 19.9; MS (EI+) *m/z* 172; HRMS calcd. for $C_8H_{12}O_4$: 172.07, Found: 172.07356. Anal calcd.: C 55.81, H 7.02, O 37.13. Found: C 53.89, H 7.49.



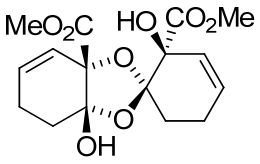
(-)-**8**

(*S*)-methyl 1-hydroxy-6-oxocyclohex-2-enecarboxylate (8).

To a solution of diol **129** (409 mg, 2.37 mmol) in dimethyl sulfoxide (7.9 mL) was added 2-iodoxybenzoic acid (1.99 g, 7.12 mmol) in portions over 1 minute. The white slurry turned to a light yellow and clear mixture over several minutes. The reaction was monitored by TLC analysis (1:1 hexanes/EtOAc, CAM) and stirred at room temperature for 15.5 hours prior to workup. The reaction mixture appeared as a slightly yellow-white slurry and was filtered. The filtrate was dropped into a stirring volume of water (18 mL) and produced a white precipitate that was stirred at room temperature for 0.5 h prior to filtration. The mother liquor was extracted with Et_2O (10 x 8 mL, 7 x 7 mL, 6 x 4 mL) and ethyl acetate (2 x 6 mL). The organic layers were combined and dried over $MgSO_4$, filtered, and concentrated provide an orange oil (585 mg crude). The crude material was subjected to silica gel chromatography (2:1 hexanes/EtOAc) and provided 240 mg (59% yield) of the α-hydroxy ketone **8** as a colorless oil that was stored for short periods of time in the freezer (< 0 ˚C).

8: R_f = 0.58 (1:1 hexanes/EtOAc); $[\alpha]_D^{20}$ = -239.7 (*c* 1.0, $CHCl_3$); FT-IR (film) ν 3468, 2957, 1744, 1725, 1438, 1259, 1138, 1040, 996, 875, 813 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 2.46 – 2.71 (m, 3 H), 2.88 – 2.95 (m, 1 H), 3.72 (s, 3 H), 4.33 (s, 1H, OH), 5.72 (dt, *J*=1.7, 9.7, 1H), 6.06 (dt, *J*=3.8, 9.7, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 205.8, 170.4,

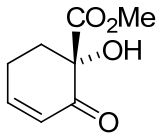
131.9, 127.6, 78.0, 53.5, 35.1, 26.9; MS (EI+) *m/z* 170; HRMS calcd. for $C_8H_{10}O_4$: 170.0600, Found: 170.0579.



(-)-idesolide (**5**)

(*1'R, 2'S, 3aR, 7aS*)-Dimethyl 2',3a-dihydroxy-3a,4,5,7a-tetrahydrospiro[benzo[*d*][1,3]dioxole-2,1'-cyclohex[3]ene]-2'7a-dicarboxylate [(-)-Idesolide (5)]. To a solution of α-hydroxy ketone **8** (45 mg, 0.261 mmol) in chloroform was added anhydrous sodium bicarbonate (44 mg, 0.522 mmol) and the heterogeneous mixture, including stir bar, was concentrated on a rotoevaporator with slow rotation in attempts to concentrate the oil at the bottom of the round bottom flask and evenly distribute the sodium bicarbonate. The resulting colorless oil, spiked with sodium bicarbonate, was subjected to slow stirring under argon atmosphere. After several hours the stirring was inhibited by the viscosity of the crude reaction mixture. The reaction mixture was allowed to stand at room temperature under argon atmosphere. NMR analysis after 43 hours indicated a 1:0.6 ratio of idesolide (**5**) to monomer **8**. The reaction was allowed to proceed for an additional 5.5 hours before the mixture was diluted with $CHCl_3$ and filtered. The filtrate was concentrated *in vacuo* to provide 54 mg of a colorless oil that was stored at -78 °C overnight. Oily white crystals were observed and the crude material was triturated with pentanes (4 x 0.2 mL) and hexanes (6 x 0.2 mL) to provide 25 mg of (-)-idesolide (**5**) (57% yield) as white crystalline solid.

5: R_f = 0.48 (1:1 hexanes/EtOAc); mp = 136-139 °C; $[\alpha]_D^{20}$ = -242.5 (*c* 1.0, $CHCl_3$); FT-IR (film) ν 3365, 3030, 2955, 2848, 1755, 1738, 1439, 1350, 1259, 1124, 975, 802, 751 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 6.01 (m, 2H), 5.61 (dd, *J* = 1.8, 10.0 Hz, 1H), 5.49 (dd, *J* = 2.5, 10.1 Hz, 1H), 3.93 (s, 3H), 3.79 (s, 3H), 2.41 (m, 2H), 2.27 (m, 4H), 2.15 (m, 1H), 1.85 (m, 1H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 173.2, 169.1, 132.5, 130.7, 126.5, 126.2, 111.0, 102.2, 86.4, 76.7, 54.3, 52.8, 31.1, 29.7, 24.4, 22.4; MS (EI+) *m/z* 340; HRMS calcd. for $C_{16}H_{20}O_8$: 340.1200, Found: 340.1150. Anal calcd.: C 56.47, H 5.92. Found: C 55.27, H 5.84.

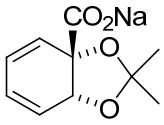


347

(*S*)-Methyl 1-hydroxy-2-oxocyclohex-3-enecarboxylate (347).

To a solution of diene **340** (65 mg, 0.381 mmol) in distilled toluene (1.5 mL) was added Grubbs First Generation catalyst (15 mg, 0.019 mmol) under argon and with stirring. The lightly colored brown/yellow solution quickly turned purple upon addition of catalyst. The reaction was heated to 111 °C and turned to a clear brown color over several minutes. The reaction was monitored by TLC analysis and deemed complete after approximately 17 h. The reaction mixture was concentrated to dryness and the crude black oil (70 mg) was chromatographed on silica gel (2:1 hexanes/EtOAc → 1:1 hexanes/EtOAc) to yield 28 mg (43% yield) of enone **347** as a light brown/red oil.

347: R_f = 0.52 (1:1 hexanes/EtOAc); FT-IR (film) ν 3460, 2955, 2938, 1741, 1681, 1436, 1389, 1259, 1220, 1198, 1139, 1114, 1070 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 7.09 (m, 1H), 6.17 (d, *J* = 10.2 Hz, 1 H), 4.24 (OH, s, 1H), 3.79 (s, 3H), 2.62 (m, 3H), 2.10 (m, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 194.8, 170.7, 152.7, 126.6, 53.0, 32.1, 24.2; MS (EI+) *m/z* 170; HRMS calcd. for $C_8H_{10}O_4$: 170.0579, Found: 170.0579.



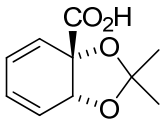
354

(*3aS,7aR*)-Methyl 2,2-dimethyl-3a,7a-dihydrobenzo[*d*][1,3]dioxole-3a-carboxylate, sodium salt (**354**).

To a slurry of mixed sodium/potassium carboxylate of **337** [61.6 g (81.1 g at 76 wt% purity), 317 mmol] in 2,2-dimethoxypropane (790 mL, 0.4 M) at 0 ˚C with vigorous stirring was added trifluoroacetic acid (146 mL, 1.90 mol, 6.0 equiv) dropwise over 6 minutes while the internal temperature was monitored; no significant exotherm observed. The reaction mixture was warmed up to room temperature and stirred until the consumption of **337** was observed by TLC (4:1 EtOAc/MeOH) and/or HPLC analysis (254 nm) at approximately 6 h. The reaction mixture was filtered through a pad of Celite and the filtrate concentrated *in vacuo* to a dark brown/black oil. The crude oily mixture was dissolved in H_2O (100 mL) and extracted twice with CH_2Cl_2 . The organic phase was then extracted with an aqueous $NaHCO_3$ solution (sat'd, 3 x 100 mL) and concentrated to dryness *in vacuo* to provide the sodium salt of **354** as a solid, contaminated with other inorganic salts. The crude product was then dissolved in MeOH and the heterogeneous

mixture containing undissolved inorganic salts was filtered. The filtrate was concentrated *in vacuo* to yield 62.1 g (90%) of sodium salt acetonide **354** as a slightly off-white solid. The storage of the sodium carboxylate was convenient in that its shelf life at room temperature exceeded several months with no loss in purity as indicated by NMR analysis.

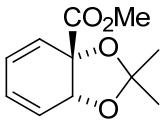
354: ^{1}H NMR (300 MHz, D_2O) δ 6.13 (dd, J = 5.6, 9.5 Hz, 1H), 6.06 (dd, J = 5.5, 9.6 Hz, 1H), 5.92 (dd, J = 4.3, 9.5 Hz, 1H), 5.71 (dd, J = 9.9, 4.5 Hz, 1H), 4.80 (d, J = 4.2 Hz, 1H), 1.33 (s, 3H), 1.33 (s, 3H).



356

The corresponding free acid was obtained by the acidification of a cold aqueous solution of the salts with 6 M HCl and extraction with EtOAc. Spectral data matches that reported in the literature.[66, 69]

(*3aS,7aR*)-2,2-Dimethyl-3a,7a-dihydrobenzo[*d*][1,3]dioxole-3a-carboxylic acid (**356**): $[\alpha]_D^{20}$ = -33.9 (c = 0.9, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.23 (bs, 1H), 6.16 (m, J = 3.6 Hz, 1H), 6.04 (dd, J = 4.7, 9.6 Hz, 1H), 5.77 (q, J = 3.2 Hz, 1H), 4.93 (d, J = 4.2 Hz, 1H), 1.49 (s, 3H), 1.44 (s, 3H).[66]

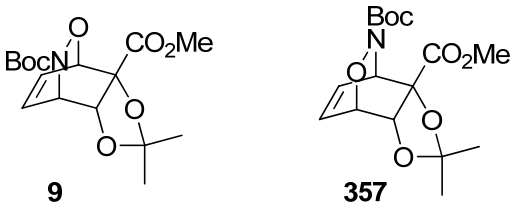


355

(*3aS,7aR*)-Methyl 2,2-dimethyl-3a,7a-dihydrobenzo[d][1,3]dioxole-3a-carboxylate (355).

To a clear light brown solution of freshly extracted **356** (67 g, 341 mmol) in methylene chloride (1.7 L, 0.2 M) at 10 ˚C was added MeOH (34 mL, 853 mmol, 2.5 equiv), dimethylaminopyridine (4.2 g, 34.1 mmol, 0.1 equiv), and *N*,*N*'-dicyclohexylcarbodiimide (77.4 g, 375 mmol, 1.1 equiv) under an atmosphere of argon and with vigorous stirring; almost immediately a white slurry was generated and the reaction was stirred to room temperature and monitored by TLC analysis (5:1 hexanes/EtOAc). After 12 h the reaction mixture was filtered and the filter cake rinsed with methylene chloride (2x). The filtrate was slightly concentrated, which allowed for additional precipitation of dicyclohexylurea and the material was filtered again. The filtrate was concentrated to dryness *in vacuo* and provided crude material (71.92 g) as a brown oil that was contaminated by dicyclohexylurea. The crude material was subjected to silica gel chromatography (5:1 → 4:1 hexanes/EtOAc) and yielded 42.2 g (67%) of known methyl ester **355** as a yellow oil that solidified to a low-melting, white crystalline solid at low temperature (5 ˚C).

355: R_f = 0.51 (hexanes/EtOAc 5:1); IR (film) ν 3043, 2989, 2934, 2846, 1753, 1735, 1454, 1434, 1380, 1371, 1254, 1209, 1168, 1036, 885, 710 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.12 (m, 2H), 6.04 (m, 1H), 5.84 (m, 1H), 4.99 (d, J = 4.1 Hz, 1H), 3.81 (s, 3H), 1.46 (s, 3H), 1.44 (s, 3H); ^{13}C ($CDCl_3$, 75 MHz) δ 172.2, 124.8, 124.5, 124.1, 124.0, 106.8, 79.4, 72.7, 53.0, 26.9, 25.2.**Error! Bookmark not defined.**66



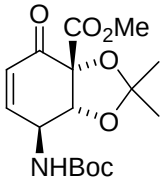
9 **357**

8-*tert*-butyl 3a-methyl (*3aR,4R,7S,7aR*)-2,2-dimethyl-7,7a-dihydro-4,7-(epoxyimino)-1,3-benzodioxole-3a,8(4*H*)-dicarboxylate (9/357).

To a solution of diene ester **355** (2.72 g, 12.9 mmol) in a mixture of MeOH/H_2O (4:1 v/v, 65 mL, 0.2 m) at 0 ˚C was added sodium periodate (3.60 g, 16.8 mmol, 1.3 equiv) as a single portion. To this mixture was added a solution of *t*-butyl hydroxycarbamate (2.24 g, 16.8 mmol, 1.3 equiv) in MeOH/H_2O (4:1 v/v, 10 mL, 1.7 M) dropwise over several minutes. The reaction mixture became a thick slurry upon addition of the hydroxy carbamate and the progress of the reaction was monitored by TLC analysis (4:1 hexanes/EtOAc, CAM). Upon consumption of **355** (approximately 2.5 h) the reaction mixture was diluted with EtOAc and the thick slurry was filtered. The filter cake was rinsed with EtOAc and the filtrate was collected and washed with aqueous $NaHCO_3$ (saturated, 1x), H_2O (1x), and then brine (1x). The organic phase was dried over Na_2SO_4, filtered and concentrated *in vacuo* to yield an orange oil. The crude material was chromatographed on silica gel (2:1 hexanes/EtOAc) to yield a mixture regioisomers **9** and **357** (4.32 g, 96%; 3:1 **9**/**357** ratio determined by NMR). The purity of the crude material was sufficient enough to use without further purification.

9/357: R_f = 0.34 (4:1 hexanes/EtOAc); IR (film, cm^{-1}) *v* 2984, 2954, 2940, 2253, 1745, 1709, 1458, 1437, 1382, 1372, 1263, 1213, 1160, 1110, 1088, 1061, 1017, 877, 732; ^1H NMR (600 MHz, $CDCl_3$) δ 6.53 (m, *J* = 4.9 Hz, 1H), 6.49 (dd, *J* = 2.0, 8.0 Hz, 1H), 5.17

(d, J = 4.4 Hz, 1H), 5.14 (dd, J = 1.4, 5.9 Hz, 1H), 5.09 (m, J = 2.9 Hz, 1H), 3.89 (s, 1H), 1.48 (s, 1H), 1.34 (s, 1H), 1.31 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.9, 157.0, 131.0, 129.9, 128.7, 112.7, 82.7, 82.3, 74.4, 72.7, 53.5, 53.1, 28.1, 26.2, 26.1; MS (EI+) *m/z* (%) 341 (0.4), 159 (12), 158 (35), 151 (7), 143 (21), 124 (8), 109 (7), 105 (13), 93 (13), 83 (22), 73 (21), 65 (12), 61 (15), 59 (42), 58 (16), 57 (100), 56 (26), 55 (16), 45 (20), 44 (34), 43 (96), 42 (17), 41 (65); HRMS (+EI) calcd for $C_{12}H_{23}N_1O_7$: 341.1475. Found: 341.1475.



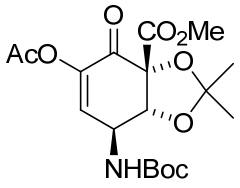
360

(*3aR,7S,7aR*)-Methyl-7-(*tert*-butoxycarbonylamino)-2,2-dimethyl-4-oxo-3a,4,7,7a-tetrahydrobenzo[*d*][1,3]dioxole-3a-carboxylate (**360**).

To a solution of isoxazolidines **9/357** (773 mg, 2.26 mmol) in MeCN/H_2O (16 mL, 0.15 M, 15:1 *v*/*v*) at 80 ˚C was added molybdenum hexacarbonyl (897 mg, 3.39 mmol, 1.5 equiv) with stirring. The reaction was monitored by TLC analysis ($CHCl_3$/MeOH 95:5) and deemed complete after 24 hours. The reaction mixture was filtered through a plug of Celite and the filtrate concentrated to dryness *in vacuo*. The crude mixture of amino alcohol regioisomers (3:1 ratio established by NMR analysis) was subjected to oxidative conditions by dissolution in methylene chloride (23 mL, 0.1 m), chilling the solution to 0 ˚C, and a single portion addition of Dess-Martin periodinane (1.44 g, 3.39 mmol, 1.5 equiv) with stirring. The reaction mixture was allowed to slowly warm to ambient

temperature and was monitored by TLC analysis (hexanes/EtOAc 1:1). After 23 h the reaction was quenched with aq. $Na_2S_2O_3$ (sat'd, 2 mL) and diluted with methylene chloride (25 mL). Precipitation occurred and the solid was filtered. The organic filtrate was washed with aq. $NaHCO_3$ (sat'd, 1 x 6 mL), brine (1 x 8 mL), and then dried over $MgSO_4$ and filtered. The crude organic solution was concentrated *in vacuo* to a solid crude material (1.34 g) that was chromatographed on silica gel (two chromatographic processes) ($CHCl_3$/MeOH/hexanes 78:4:1) to yield **360** (246 mg, 32% from **9**/**357**) as a yellow-orange oil and **361** (30 mg, 4% from **9**/**357**) as a yellow oil.

360: R_f = 0.60 (hexanes/EtOAc 1:1); $[\alpha]_D^{20}$ = 64.9 (c = 1.0, $CHCl_3$); IR (film) *v* 3029, 2986, 1710, 1692, 1496, 1299, 1226, 1162, 1101, 770 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.87 (ddd, *J* = 1.6, 5.0, 9.9 Hz, 1H), 6.86 (d, *J* = 1.7 Hz, 1H), 6.16 (d, *J* = 10.2 Hz, 1H), 5.41 (d, *J* = 9.3 Hz, 1H), 4.84 (dd, *J* = 5.0, 7.4 Hz, 1H), 4.43 (s, 1H), 3.83 (s, 1H), 1.44 (s, 1H), 1.34 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 191.3, 168.6, 154.7, 145.8, 127.5, 111.2, 82.0, 80.6, 79.4, 77.3, 53.6, 45.4, 28.3, 27.1, 25.6; MS (+EI) *m/z* (%) 285 (2), 270 (4), 248 (7), 228 (9), 159 (55), 143 (7), 127 (41), 96 (9), 83 (37), 73 (21), 59 (15), 57 (100), 43 (18), 41 (18); HRMS (+EI) calcd for $C_{16}H_{23}O_7N$: 341.1475. Found: 341.1481.



365

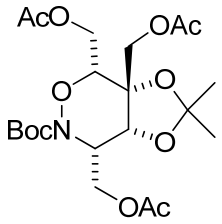
(*3aR,7S,7aR*)-Methyl-7-(*tert*-butoxycarbonylamino)-5,6-dihydroxy-2,2-dimethyl-4-oxohexahydrobe-nzo[*d*][1,3]dioxole-3a-carboxylate (**365**).

Step 1. To a solution of sodium periodate (904 mg, 4.22 mmol, 1.5 equiv.) in H_2O (0.85 mL, 3.3 M) at room temperature was added cerium (III) chloride heptahydrate (104 mg, 0.281 mmol, 10 mol%) and the white slurry was gently heated so that it became yellow in color. The yellow slurry was cooled in an ice-bath and a mixture of ethyl acetate/acetonitrile (1.2:1 v/v) was added. To this chilled mixture was added ruthenium (III) chloride hydrate ($RuCl_3 \cdot xH_2O$, 0.25 mol%) and the reaction mixture immediately turned to a dark brown color. A solution of enone **360** (962 mg, 2.81 mmol, 1.0 equiv.) in ethyl acetate (6.5 mL, 0.43 M) was added to the reaction mixture and the reaction mixture was stirred for approximately 23 hours with gradual warming to room temperature over the first 1.5 hours. The reaction was monitored by TLC analysis (ethyl acetate/hexanes 2:1). Once the reaction was deemed complete by TLC small portions of sodium sulfite were added until no more active oxidant was detectable by KI/starch paper. The reaction mixture was filtered and the filtered solid washed with small portions of ethyl acetate. The filtrate was concentrated to yield 1.00 g of crude material as a light yellow foam-like solid. Silica gel chromatography (ethyl acetate/hexanes 2:1) provided 647 mg of **352** (quant.).

Step 2. To a solution of reasonably pure **352** (26 mg, 0.081 mmol, 1.0 equiv.) in methylene chloride (0.8 mL, 0.1 M) at room temperature was added acetic anhydride (30 μL, 0.327 mmol, 4.0 equiv.), triethylamine (45 μL, 0.327 mmol, 4.0 equiv.), and dimethylaminopyridine (DMAP, cat.) in succession. Upon addition of triethylamine the clear and colorless mixture became clear and yellow and following the addition of DMAP the mixture lightened in color. The reaction was monitored by TLC analysis (hexanes/ethyl acetate 1:1) and starting material was consumed after 10 minutes. The

reaction was quenched with an aqueous solution of ammonium chloride (sat'd, 0.2 mL) and followed by the addition of H_2O (0.4 mL). The phases were separated and the organic phase was washed with H_2O (2x), brine (1x), and then dried over $MgSO_4$. The organic phase was concentrated *in vacuo* to yield 28 mg of crude material as a white foam-like solid. The crude material was subjected to chromatography on silica gel (hexanes/ethyl acetate 2:1) to yield 18 mg (52%) of acetate **365** as a white solid.

365: mp 103 – 105 ˚C (pentane); R_f = 0.54 (hexane/EtOAc 2:1); $[\alpha]_D^{20}$ = +64.9 (c = 0.9, $CHCl_3$); IR (film) *v* 3393, 2984, 1773, 1703, 1498, 1369, 1303, 1193, 1165, 1094, 1014, 875, 755 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.55 (d, *J* = 5.6 Hz, 1H), 5.48 (d, *J* = 9.6 Hz, 1H), 5.03 (dd, *J* = 9.1, 6.0 Hz, 1H), 4.46 (s, 1H), 3.89 (s, 3H), 2.25 (s, 3H), 1.53 – 1.46 (m, 12H), 1.45 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 185.2, 168.1, 154.6, 143.9, 131.5, 111.9, 83.4, 80.7, 79.5, 77.4, 77.0, 76.6, 53.8, 44.9, 28.3, 27.0, 25.6, 22.3, 20.2, 14.0; HRMS (EI+ TOF) calcd for $C_{18}H_{25}NO_9$: 399.1529. Found: 399.1539.



368

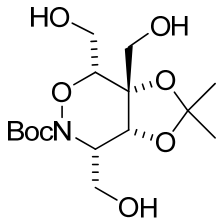
[(*3aR,4R,7S,7aR*)-2,2-Dimethyldihydro-4*H*-[1,3]dioxolo[4,5-*d*][1,2]oxazine-3a,4,7-triyl]tri(methylene) triacetate (**368**).

To a clear and colorless solution of isoxazolidine **9/357** (104 mg, 0.295 mmol, ~3:1 mixture of regioisomers) in methylene chloride (6 mL) at -70 °C was bubbled ozone until

a blue color persisted (*ca*. 35 minutes) which corresponded to the consumption of **9/357** by TLC analysis (hexanes/EtOAc 2:1). Oxygen was used to purge the reaction mixture and make it devoid of excess ozone and its blue color. Sodium borohydride (21 mg) was added directly to the reaction mixture at -54 °C and the reaction was slowly warmed to room temperature and allowed to stir for a total of 23 h at room temperature. To the hazy, off-white reaction mixture was added H_2O (3 drops) and stirring was allowed for several minutes prior to concentration *in vacuo*. The crude mixture was then reconstituted in methylene chloride (3 mL) and acetic anhydride (140 μL, 1.47 mmol), triethylamine (205 μL, 1.47 mmol), and 4-dimethylaminopyridine (DMAP, cat.) was added in sequence and allowed to stir overnight at room temperature. After 17 hours the reaction was quenched with aq. NH_4Cl (sat'd, 1 mL) and the phases were separated. The organic phase was washed with H_2O (2 x 1 mL) and brine (1 x 1 mL), dried over anhydrous $MgSO_4$, and concentrated *in vacuo* to provide an orange oil (119 mg). The crude material was chromatographed on silica gel (hexanes/EtOAc 2:1) to yield **368** (31 mg, 22%) as a colorless oil.

368: R_f = 0.41 (hexanes/EtOAc 2:1); $[\alpha]_D^{20}$ = +37.5° (*c* 0.7, $CHCl_3$); IR (film) υ 2984, 2936, 1746, 1706, 1456, 1370, 1337, 1233, 1165, 1132, 1043, 930, 885, 854, 759 cm^{-1}; ^1H NMR (300 MHz, DMSO) δ 4.55 (d, J = 4.0 Hz, 1H), 4.38 – 4.23 (m, 3H), 4.20 (s, 2H), 4.14 (dd, J = 8.9, 6.6 Hz, 1H), 4.01 (dd, J = 13.0, 8.4 Hz, 1H), 3.81 (dd, J = 8.3, 2.1 Hz, 1H), 2.04 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H), 1.45 (s, 9H), 1.40 (s, 3H), 1.35 (s, 3H). ; ^{13}C NMR (75 MHz, DMSO) δ 170.7, 170.5, 170.1, 155.9, 109.6, 81.9, 81.8, 78.3, 73.5, 64.8, 62.2, 62.0, 52.8, 28.3, 27.2, 26.0, 21.2, 21.1, 20.9; MS (+EI) *m/z* (%) 283 (M^+-CH_3, 1), 375 (11), 223 (21), 167 (20), 149 (100), 101 (15), 57 (87); HRMS (+EI) calcd for

$C_{21}H_{33}O_{11}N$: 475.2054. Found 475.2054. Anal. Calcd for $C_{21}H_{33}O_{11}N$: C, 53.05; H, 7.00. Found C, 52.84; H, 6.87.



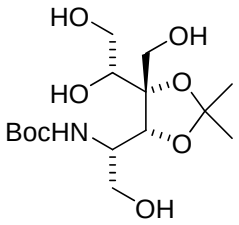
366

(*3aR,4R,7S,7aR*)-*tert*-Butyl3a,4,7-tris(hydroxymethyl)-2,2-dimethyldihydro-3a*H*-[1,3]dioxolo[4,5-*d*][1,2]oxazine-6(4*H*)-carboxylate (366).

To a solution of protected isoxazolidine **368** (39 mg, 0.082 mmol) in MeOH (2 mL) at room temperature was added an aqueous solution of potassium carbonate (10%, 1.3 mL) with stirring. The consumption of **368** was observed by TLC analysis (EtOAc) after 1.2 hours and the reaction mixture was neutralized with aqueous HCl (1 M). The mixture was extracted with EtOAc (3 x 1.5 mL), the combined organic phases were dried over anhydrous sodium sulfate and concentrated *in vacuo* to yield **366** (31 mg, quant.) as a colorless oil. An analytical sample was obtained by silica gel chromatography (EtOAc).

366: R_f = 0.37 (EtOAc); $[\alpha]_D^{20}$ = +56.7° (*c* = 0.7, $CHCl_3$); IR (film, cm^{-1}) υ 3379, 2983, 2935, 1700, 1456, 1393, 1370, 1337, 1251, 1217, 1150, 1117, 1050, 1010, 904, 866, 844, 826, 756; ^1H NMR (600 MHz, DMSO) δ 5.18 (t, *J* = 5.2 Hz, 1H), 4.85 (t, *J* = 5.5 Hz, 1H), 4.52 (d, *J* = 3.6 Hz, 1H), 4.48 (t, *J* = 5.7 Hz, 1H), 3.79 (dt, *J* = 9.8, 4.2 Hz, 1H), 3.68 – 3.63 (m, *J* = 9.5, 4.7 Hz, 1H), 3.63 – 3.53 (m, 4H), 3.45 – 3.38 (m, 2H), 1.42 (s, 9H),

1.34 (s, 3H), 1.29 (s, 3H); ^{13}C NMR (150 MHz, DMSO) δ 157.4, 108.3, 85.1, 81.1, 80.1, 74.5, 63.8, 60.4, 59.1, 57.5, 28.4, 27.5, 26.6; MS (FAB+) *m/z* (%) 350 (3), 294 (13), 250 (10), 192 (14), 136 (12), 109 (11), 107 (12), 97 (14), 95 (17), 57 (100), 43 (58), 41 (50), 39 (18), 29 (23); HRMS (FAB+) calcd for $C_{15}H_{28}O_8N$: 350.1815 [M^++1]. Found 350.1791. Anal. Calcd for $C_{15}H_{27}O_8N$: C, 51.57; H, 7.79. Found C, 51.33; H, 7.63.



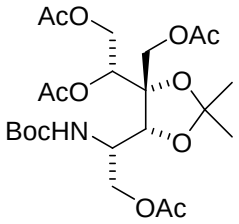
370

***tert*-Butyl (*S*)-1-((*4R,5S*)-5-((*R*)-1,2-dihydroxyethyl)-5-(hydroxymethyl)-2,2-dimethyl-1,3-dioxolan-4-yl)-2-hydroxyethylcarbamate** (**370**).

To a clear, light yellow and degassed solution of isoxazolidine **366** (152 mg, 0.435 mmol, 1.0 equiv) in MeCN/H_2O (4.4 mL, 0.1 M, 15:1 *v*/*v*) at room temperature was added molybdenum hexacarbonyl (sublimed, 252 mg, 0.957 mmol, 2.2 equiv.) with stirring and under a blanket of argon. The reaction mixture slowly changed from a light yellow heterogeneous mixture of $Mo(CO)_6$ crystals and dissolved substrate to a darker homogeneous mixture and an eventual black mixture upon heating to reflux. The reaction was monitored by TLC analysis (4:1 EtOAc/MeOH, CAM) and stirred for *ca*. 20 h prior to cooling to room temperature and spiking the reaction mixture with solid $NaHCO_3$ (two small spatula tips) and several drops of an aqueous $NaHCO_3$ (sat'd) solution and exposing to air for > 8 h. Celite (small scoop) was added to the reaction mixture with stirring and then passed over a short pad of Celite/SiO_2 (1:1 *w*/*w*) and eluted with EtOAc.

Three fractions were collected and the main fraction that contained **370** was concentrated *in vacuo* to yield 133 mg (87%) of **370** as a slightly pink crude material that was used without further purification.

370: mp 61 – 63 ˚C (EtOAc); R_f = 0.58 (EtOAc/MeOH 4:1); $[\alpha]_D^{20}$ = +2.1 (*c* = 1.5, $CHCl_3$); IR (film, cm^{-1}) ν 3418, 2982, 2936, 1691, 1507, 1456, 1384, 1252, 1218, 1168, 1053, 894, 865, 615; ^1H NMR (300 MHz, MeOD) δ 4.38 (d, *J* = 4.6 Hz, 1H), 4.11 (dd, *J* = 11.0, 5.3 Hz, 1H), 3.88 (dd, *J* = 7.3, 3.5 Hz, 1H), 3.81 – 3.72 (m, 1H), 3.72 – 3.57 (m, 5H), 1.51 (s, 3H), 1.47 (s, 8H), 1.40 (s, 3H); ^{13}C NMR (75 MHz, MeOD) δ 156.7, 107.5, 84.2, 79.0, 78.4, 71.6, 63.6, 62.3, 62.2, 51.0, 27.3, 25.8, 25.1; MS (FAB+) *m*/*z* (%) 353 (12), 352 (67.5), 296 (17), 253 (12), 252 (99), 238 (15), 214 (21), 194 (33), 176 (10), 149 (29), 113 (12), 57 (100), 55 (13), 43 (28), 41 (24), 29 (12); HRMS (FAB+) calcd for $C_{15}H_{30}NO_8$: 352.1971 [M+1]. Found 352.1976; Anal. Calcd for $C_{15}H_{29}O_8N$: C, 51.27; H, 8.32. Found C, 51.37; H, 8.16.



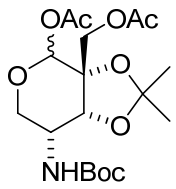
370a

((*4S,5R*)-5-((*S*)-2-Acetoxy-1-(*tert*-butoxycarbonylamino)ethyl)-4-((*R*)-2-acetoxy-1-hydroxyethyl)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl acetate (**370a**).

To a clear and colorless solution of isoxazolidine triol **366** (52 mg, 0.148 mmol) in a mixture of acetonitrile/H_2O (1.5 mL, 15:1 v/v) at room temperature was added

molybdenum hexacarbonyl (58 mg, 0.223 mmol) with stirring. The reaction mixture was heated to reflux for *ca*. 24 hours prior to an additional portion of molybdenum hexacarbonyl (39 mg, 0.148 mmol) was added. The reaction mixture was stirred at reflux for another 12 hours prior to cooling to room temperature and filtration through a pad of Celite. The filtrate was concentrated *in vacuo* to yield a crude black mass that was dissolved in DCM (2.5 mL) and acetic anhydride (140 μL, 1.48 mmol), Et_3N (206 μL, 1.48 mmol), and 4-dimethylaminopyridine (cat.) was added sequentially at room temperature and with stirring. The reaction mixture was stirred for 20 h and quenched with aq. NH_4Cl (sat'd, 0.5 mL). The phases were separated and the organic phase was washed with H_2O (2x) and brine (1x). The organic phase was dried over $MgSO_4$ and concentrated to provide crude material (25 mg) that was chromatographed on silica gel (hexanes/EtOAc 1:1) to yield **370a** (6 mg, 8%) as a white foam.

370a: R_f = 0.53 (hexanes/EtOAc 1:1); IR (film) υ 3368, 2981, 2930, 1747, 1715, 1497, 1454, 1369, 1226, 1165, 1046, 870, 603 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.39 (dd, *J* = 7.2, 3.4 Hz, 1H), 4.96 (d, *J* = 9.0 Hz, 1H), 4.34 – 4.23 (m, 2H), 4.21 (d, *J* = 4.2 Hz, 2H), 4.18 – 4.05 (m, 3H), 2.13 (s, 3H), 2.09 (s, 3H), 2.05 (s, 3H), 1.99 (s, 3H), 1.50 (s, 3H), 1.46 (s, 8H), 1.36 (s, 3H); MS (EI+) *m/z* (%) 448 (2), 404 (3), 346 (10), 287 (14), 259 (15), 149 (11), 102 (36), 57 (64), 43 (100); HRMS (EI+) calcd for $C_{22}H_{34}O_{12}N$: 504.2081 [M^+-CH_3]. Found 504.2084. Anal. Calcd for $C_{23}H_{37}O_{12}N$: C, 53.17; H, 7.18. Found C, 54.20; H, 6.69.



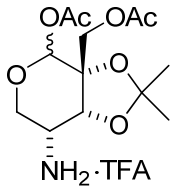
373

((*3aR,7S,7aR*)-4-Acetoxy-7-(*tert*-butoxycarbonylamino)-2,2-dimethyltetrahydro-3a*H*-[1,3]dioxolo[4,5-*c*]pyran-3a-yl)methyl acetate (**373**).

To a clear and red solution of tetrol **370** (2.37 g, 6.74 mmol; 3.70 g **370** with 64.1% *w*/*w* purity) in methylene chloride (70 mL, 0.1 M) at 0 ˚C was added a freshly prepared quantity of silica gel support $NaIO_4$ (15% *w*/*w*; 13.5 g, 9.43 mmol, 1.4 equiv)[1] over 2 minutes; the color of the slurry immediately changed from red to light yellow and a small exotherm was detected. The reaction was monitored by TLC analysis (EtOAc, CAM) and **370** was consumed after 0.5 h. The reaction mixture was filtered and the solid support was rinsed thoroughly with methylene chloride and ethyl acetate until no chemical species was detectable by TLC [R_f = 0.61 (EtOAc)]. The clear and yellow filtrate was dried over Na_2SO_4 and quickly concentrated *in vacuo* prior to reconstitution in methylene chloride (70 mL, 0.1 M) and addition of acetic anhydride (4.5 mL, 47.1 mmol, 7 equiv), triethylamine (6.6 mL, 47.1 mmol, 7 equiv), and a catalytic amount of dimethylamino pyridine with stirring. The acylation was monitored by TLC analysis (EtOAc, CAM) and the intermediate lactol **372** (R_f = 0.61) was consumed within 1 h. An aqueous solution of NH_4Cl (half sat'd, 25 mL) was added to the chilled (ice bath) reaction mixture. After warming to ambient temperature the phases were separated and the organic phase was washed with H_2O (1x), brine (1x), dried over Na_2SO_4, filtered and then concentrated *in vacuo* to an orange oil (4.03 g). The crude material was chromatographed on silica gel

(3:1 → 2:1 hexanes/EtOAc) to yield 1.53 g (56%) of acetal **373** as a hygroscopic white foam.

373: mp 53 – 56 ˚C (pentane); R_f = 0.39 (2:1 hexanes/EtOAc); IR (film) υ 3362, 2983, 2934, 1764, 1747, 1711, 1511, 1454, 1382, 1366, 1305, 1240, 1218, 1171, 1097, 1053, 1006, 899, 872, 757, 735 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.69 (s, 1H), 5.27 (d, *J* = 7.8 Hz, 1H), 4.48 – 4.31 (m, *J* = 4.0, 2.0 Hz, 3H), 4.09 (d, *J* = 12.1 Hz, 1H), 4.03 (s, 1H), 3.86 (d, *J* = 12.2 Hz, 1H), 2.15 (s, 3H), 2.13 (s, 3H), 1.53 (s, 3H), 1.46 (s, 8H), 1.41 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.1, 169.0, 154.9, 109.7, 95.5, 80.1, 66.6, 61.5, 46.8, 28.3, 27.8, 26.3, 20.9, 20.8; MS (EI+) *m/z* (%) 403 (< 1), 272 (19), 227 (20), 184 (18), 113 (45), 101 (12), 88 (29), 57 (76), 43 (100); HRMS (EI+) calcd for $C_{18}H_{29}O_9N$: 403.1842 [M^+]. Found 403.1842. Anal. Calcd for $C_{18}H_{29}O_9N$: C, 53.59; H, 7.25. Found C, 53.68; H, 7.26.



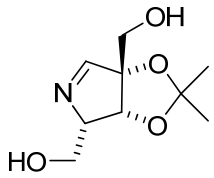
11

((*3aR,7S,7aR*)-4-Acetoxy-7-amino-2,2-dimethyltetrahydro-3aH-[1,3]dioxolo[4,5-c]pyran-3a-yl)methyl acetate (**11**).

To a flamed dried flask a solution of acetal **373** (33 mg, 0.081 mmol) in methylene chloride (2 mL) at 0 ˚C was added trifluoroacetic acid (0.5 mL) as a single portion with stirring and under a blanket of argon. The reaction mixture was allowed to warm to room temperature and monitored by TLC analysis (EtOAc/MeOH 4:1 (or) hexanes/EtOAc

2:1). The reaction was deemed complete after 50 minutes and concentrated *in vacuo* to provide crude material (31 mg) that was passed through a plug of deactivated (20% wt/wt H_2O) silica gel and eluted with ethyl acetate. The mother liquor was concentrated to yield 15 mg (62%) of amine salt **11** as a white crystalline solid.

11: mp 163 – 165 ˚C (Et_2O); R_f = 0.13 (ethyl acetate); $[\alpha]_D^{20}$ = -17.9 (c = 0.6, $CHCl_3$); IR (KBr, $cm^{-1)}$ *v* 3369, 2991, 2939, 1769, 1746, 1678, 1376, 1212, 1137, 1063, 907, 836, 799, 721, 665; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.85 (bs, 2H), 5.68 (s, 1H), 4.68 (d, *J* = 12.8 Hz, 1H), 4.44 (s, 1H), 4.36 (d, *J* = 12.8 Hz, 1H), 4.20 – 3.90 (m, 2H), 3.60 (s, 1H), 2.15 (s, 2H), 2.14 (s, 2H), 1.54 (s, 3H), 1.42 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.2, 168.9, 110.1, 95.5, 77.8, 77.4, 77.0, 76.6, 75.6, 65.6, 60.8, 47.6, 27.8, 26.1, 20.8, 20.7; HRMS (EI+) calcd for $C_{13}H_{21}NO_7$: 303.1318. Found: 303.1339 Anal. Calcd for $C_{15}H_{22}F_3NO_9$: C, 43.17; H, 5.31. Found C, 42.92; H, 5.34.



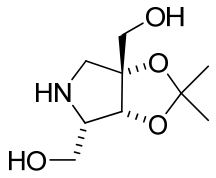
374

((*3aR,6S,6aR*)-2,2-Dimethyl-6,6a-dihydro-3aH-[1,3]dioxolo[4,5-c]pyrrole-3a,6-diyl)dimethanol (**374**).

To a flamed dried vessel that contained vacuum dried solid K_2CO_3 (31 mg, 0.229 mmol, 4.0 equiv) was added a solution of trifluoroacetate salt **11** (23 mg, 0.057 mmol, 1.0 equiv) in anhydrous MeOH-d_4 (0.50 mL, 0.11 M) with stirring. The reaction mixture was stirred for 5.5 h at room temperature and the reaction was monitored by ^{1}H NMR [Note: R_f is

identical to that of starting material **11**, R_f = 0.52 (4:1 EtOAc/MeOH)]. The reaction was filtered through a pad of Celite and washed with small volumes of MeOH (2 x 0.5 mL). The filtrate was concentrated *in vacuo* to provide an oily white crystalline material (32 mg) that was contaminated with KOAc and CF_3CO_2K. The crude material could be purified by trituration with acetone several times to yield 9 mg of **374** (81%) as a colorless oil.

374: R_f = 0.52 (4:1 EtOAc/MeOH); $[\alpha]^{20}_D$ = -24.4 (c = 0.45, MeOH); IR (KBr) ν 3306, 2987, 2930, 2846, 1626, 1572, 1454, 1373, 1240, 1215, 1182, 1090, 1049, 900, 863 cm^{-1}; ^1H NMR (600 MHz, MeOH-d_4) δ 4.62 (d, J = 4.1 Hz, 1H), 4.07 – 3.99 (m, 1H), 3.93 – 3.84 (m, 2H), 3.84 – 3.77 (m, 2H), 1.39 (s, 3H), 1.35 (s, 3H); ^{13}C NMR (MeOH-d_4, 150 MHz) δ 169.2, 111.9, 97.7, 80.0, 75.6, 61.4, 59.9, 26.2, 25.5; HRMS (EI+) calcd for $C_9H_{16}NO_4$ (M+H): 202.1079. Found: 202.1071.



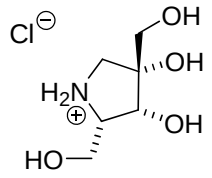
375

((*3aR,6S,6aR*)-2,2-Dimethyltetrahydro-3aH-[1,3]dioxolo[4,5-c]pyrrole-3a,6-diyl)dimethanol (375).

To a round bottom flask that contained crude pyrroline **374** (22 mg, 0.109 mmol, 1.0 equiv; contaminated with KOAc/CF_3CO_2K) was added HOAc (1.0 mL; aqueous, 3 m) followed by 10% Pd/C (2 mg, 10 wt%) and the reaction vessel was purged with H_2 (3x) and the reaction mixture set to stir at room temperature for approximately 15 h under 1

atmosphere of hydrogen. The reaction mixture was filtered over a pad of Celite and rinsed with small amounts of H_2O. The filtrate was concentrated *in vacuo* to yield crude material that was crystalline in nature and was triturated with acetone (precipitating any $KOAc/CF_3CO_2K$). The mother liquor was concentrated *in vacuo* to yield a sticky white foam, 8 mg, **375** (quantative over 2 steps from TFA salt **11**).

375: R_f = 0.30 (4:1 EtOAc/MeOH); $[\alpha]^{20}_D$ = +17.3 (c = 0.40, MeOH); IR (KBr) ν 3416, 2937, 1681, 1563, 1414, 1384, 1206, 1139, 1051, 803, 723 cm^{-1}; ^1H NMR (600 MHz, MeOH-d_4) δ 4.60 (d, J = 3.0 Hz, 1H), 3.92 (dd, J = 11.4, 5.7 Hz, 1H), 3.80 (dd, J = 11.4, 7.6 Hz, 1H), 3.77 – 3.72 (m, 2H), 3.15 (dd, J = 12.8 Hz, 2H), 1.52 (s, 3H), 1.39 (s, 3H), signal (1H) under solvent peak; ^{13}C NMR (151 MHz, MeOH-d_4) δ 111.8, 91.4, 81.8, 65.0, 63.5, 58.3, 53.3, 48.0, 47.9, 47.7, 47.6, 47.5, 47.3, 47.2, 25.8, 24.8; HRMS (ESI) calcd for $C_9H_{17}NO_4$ (M+H): 204.1228. Found: 204.1236.



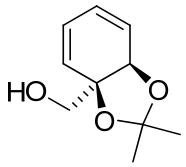
6

(*2S,3R,4R*)-3,4-Dihydroxy-2,4-bis(hydroxymethyl)pyrrolidinium chloride (**6**).

To a light yellow solution of protected pyrrolidine **375** (165 mg, 0.811 mmol) in MeOH (4 mL, 0.2 M) at room temperature was added conc. HCl (0.7 mL) with stirring and under a blanket of argon. The reaction mixture was heated to 40 ˚C and monitored by NMR. Starting acetonide **375** was consumed after approximately 5 h and the reaction mixture was concentrated *in vacuo* to dryness. The reaction mixture was reconstituted in a

minimal amount of MeOH and precipitated with the addition of Et_2O while stirring. The slightly off-white precipitate was dried under high vacuum to yield 121 mg (75%) of pyrrolidine tetrol **6** as the hydrochloride salt. An analytical sample was obtained by recrystallization from boiling MeOH to give white crystalline solid.

6: R_f = 0.0 (EtOAc/MeOH 4:1); mp 141 – 143 ˚C (MeOH); $[\alpha]_D^{20}$ = -29.9 (c = 0.95, MeOH); IR (film) *v* 3214, 1607, 1415, 1322, 1251, 1196, 1119, 1031, 696, 636, 524 cm^{-1}; ^1H NMR (600 MHz, MeOD) δ 4.26 (d, *J* = 6.7 Hz, 1H), 3.97 – 3.87 (m, 2H), 3.81 (dd, *J* = 12.7, 6.6 Hz, 1H), 3.61 (d, *J* = 11.6 Hz, 1H), 3.59 (d, *J* = 12.6 Hz, 1H); ^{13}C NMR (150 MHz, MeOD) δ 78.9, 70.7, 63.4, 63.3, 58.2, 49.9; MS (FAB+) *m/z* (%) 223 (18), 164 (56), 131 (100), 75 (11), 61 (14), 57 (23), 43 (12), 39 (45), 29 (13); HRMS (FAB+) calcd for $C_{12}H_{14}O_4N$: 164.0917. Found: 164.0930. Anal. Calcd for $C_6H_{15}NO_{4.5}Cl$: C, 34.54; H, 7.27. Found C, 34.48; H, 6.89.



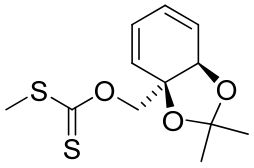
377

(*3aR,7aR*)-2,2-Dimethyl-3a,7a-dihydrobenzo[d][1,3]dioxol-3a-yl)methanol (377).

To a chilled solution of diene ester **355** (2.30 g, 9.65 mmol) in THF (65 mL) was added $LiBH_4$ (252 mg, 11.5 mmol, 1.2 equiv) as a single portion and with stirring. The reaction mixture was allowed to warm to room temperature and was monitored by TLC analysis (hexanes/EtOAc 1:1). Upon consumption of **355** the reaction mixture was cooled to 0 ˚C and quenched with H_2O (10 mL) over 2 mintues. The quenched mixture was filtered and

the filtrate concentrated *in vacuo* to remove THF. The aqueous mixture was extracted with EtOAc (2x) and the organic phases were then washed with H_2O (1x), brine (1x), and then dried over Na_2SO_4. The organic phase was concentrated *in vacuo* to a clear and colorless oil that crystallized on standing at room temperature. The oily crystals were triturated with pentanes, the mother liquor decanted, and the crystalline **377** dried under vacuum to yield 1.46 g (83%) of white crystalline solid that required no further purification.[69]

377: R_f = 0.39 (hexanes/EtOAc 1:1); mp 38 – 40 °C (hexanes/EtOAc); IR (film) 3462, 3043, 2987, 2934, 2868, 1456, 1414, 1380, 1370, 1243, 1213, 1168, 1145, 1119, 1034, 909, 864, 711 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 6.15-6.10 (m, 1H), 6.07-6.02 (m, 2H), 5.71 (d, *J* = 9.60 Hz, 1H), 4.52 (d, *J* = 4.47 Hz, 1H), 3.61 (dd, *J* = 4.48, 11.65 Hz, 1H), 3.38 (dd, *J* = 9.03, 11.68 Hz, 1H), 1.89 (dd, *J* = 4.56, 9.00 Hz, 1H), 1.48 (s, 3H), 1.40 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 128.9, 125.3, 124.4, 123.5, 106.4, 80.5, 70.9, 64.2, 27.1, 26.7; MS (+EI) *m/z* (%) 183 (3), 169 (5), 151 (27), 135 (9), 97 (9), 95 (21), 94 (21), 93 (34), 77 (13), 69 (12), 66 (11), 65 (27), 60 (40), 57 (12), 55 (18), 45 (54), 44 (96), 43 (100).



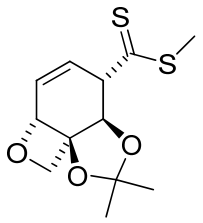
382

O-((*3aR,7aR*)-2,2-Dimethyl-3a,7a-dihydrobenzo[*d*][1,3]dioxol-3a-yl)methyl S-methyl carbonodithioate (**382**).

To a clear and colorless solution of alchol **377** (52 mg, 0.285 mmol) in THF (3 mL, 0.1 M) at -62 ˚C was added carbon disulfide (68 μL, 1.14 mmol, 4.0 equiv) with stirring. To this chilled mixture was added a solution of 0.5 M potassium hexamethyldisilazane in toluene (1.2 mL, 0.570 mmol, 2.0 equiv) over 1 minute; the reaction mixture turned from clear/colorless to clear/dark yellow-orange and was stirred at decreased temperature (< -52 ˚C) for 27 minutes before adding methyl iodide (141 μL, 2.28 mmol, 8.0 equiv) over 40 seconds. The reaction mixture slowly turned bright yellow and hazy and starting material was consumed after 20 minutes based on TLC analysis (hexanes/EtOAc 4:1, CAM); reaction mixture at -42 ˚C. The reaction was quenched with aqueous ammonium chloride (sat'd, 3 mL) at -32 ˚C and was allowed to stir to room temperature. The phases were separated and the aqueous phase extracted with EtOAc (3x). The organic phase was then washed with a small amount of H_2O (1x), brine (1x), was dried over Na_2SO_4, filtered and concentrated *in vacuo* to an orange oil, 74 mg. The crude material was chromatographed on silica gel (hexanes/EtOAc 8:1 → 4:1) to yield xanthate **382** (63 mg, 82%) as an orange oil.

382: R_f = 0.65 (hexanes/EtOAc 4:1); $[\alpha]_D^{20}$ = -296.1 (c = 1.1, $CHCl_3$); IR (film) *v* 2986, 2932, 1413, 1370, 1230, 1213, 1072 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 6.16 (dd, *J* = 5.6, 9.4 Hz, 1H), 6.07 (m, 2H), 5.77 (d, *J* = 9.5 Hz, 1H), 4.62 (d, *J* = 11.3 Hz, 1H), 4.52 (d, *J* = 11.3 Hz, 2H), 4.52 (d, *J* = 4.6 Hz, 2H), 2.58 (s, 3H), 1.49 (s, 3H), 1.41 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 215.7, 127.7, 125.3, 124.4, 123.5, 106.8, 78.2, 74.5, 71.9, 27.1, 26.4, 19.1;MS (+EI) *m/z* (%) 272 (5), 164 (23), 151 (13), 149 (20), 121 (10), 108 (13), 107 (100), 106 (30), 94 (19), 93 (32), 91 (49), 79 (39), 78 (33), 77 (35), 65 (17), 60 (47), 58 (20), 48 (23), 47 (34), 45 (20), 43 (81); HRMS (+EI) calcd for $C_{12}H_{16}O_3S_2$: 272.0541.

Found: 272.0535; Anal. Calcd for $C_{12}H_{16}O_3S_2$: C, 52.91; H, 5.92. Found C, 52.98; H, 5.88.



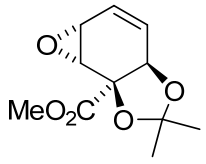
384

Methyl (*1S,4R,7S,8R*)-10,10-dimethyl-3.9.11-trioxatricyclo[6.3.0.0]undec-5-ene-7-carbodithioate (384).

To a clear and orange, degassed solution of xanthate **382** (59 mg, 0.215 mmol) in benzene (1 mL) was added tributyltin hydride (145 μL, 0.539 mmol, 2.5 equiv) and the reaction mixture was warmed. AIBN (2 mg) was added and the reaction mixture was heated to reflux with stirring. The reaction was monitored by TLC analysis (hexanes/EtOAc 4:1) until consumption of **382** was observed (ca. 6 h). The reaction was cooled to room temperature and the yellow-white slurry was adsorbed onto Celite and the solvent was removed *in vacuo*. The mixture was purified by silica gel chromatography (hexanes/EtOAc 7:1 → 4:1) and 3 mg of **384** (5%) was obtained as a light yellow oil.

384: R_f = 0.3 (hexanes/EtOAc 4:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.13 (dd, *J* = 5.0, 9.8 Hz, 1H), 6.03 (dd, *J* = 5.3, 9.8 Hz, 1H), 5.78 (d, *J* = 9.6 Hz, 1H), 5.32 (s, 1H), 4.47 (d, *J* = 4.6 Hz, 1H), 3.35 (d, *J* = 10.5 Hz, 1H), 3.18 (d, *J* = 10.4 Hz, 1H), 1.54 (s, 1H), 1.35 (s, 1H); ^{13}C (150 MHz, acetone-d_6) δ 133.3, 126.3, 109.6, 103.1, 86.1, 85.8, 72.1, 49.7, 38.7, 31.4, 25.8, 25.7, 22.4, 12.0; MS (+EI) *m/z* (%) 272 (30), 164 (31), 151 (14), 150 (10), 149 (36), 143 (20), 139 (11), 137 (11), 135 (11), 133 (9), 123 (9), 122 (8), 121 (15), 118 (11),

113 (17), 111 (15), 110 (13), 109 (18), 108 (12), 107 (78), 106 (32), 105 (22), 97 (40), 94 (11), 93 (22), 91 (32), 89 (9), 85 (11), 84 (14), 79 (40), 78 (28), 77 (48), 76 (11), 75 (35), 71 (11), 69 (12), 65 (18), 61 (11), 59 (11), 58 (22), 57 (12), 55 (12), 53 (10), 51 (14), 48 (15), 47 (26), 45 (34), 43 (100), 41 (19).

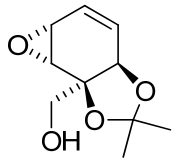


395

Methyl (*3aR,5aR,6aR,6bR*)-2,2-dimethyl-5a,6a-dihydrooxireno[*e*][1,3]benzodioxole-6b(3a*H*)-carboxylate (395).

To a solution of bromo lactone **397** (1.01 g, 3.67 mmol) in anhydrous MeOH (36 mL, 0.1 M) at room temperature was added a solution of NaOMe (198 mg, 3.67 mmol) in anhydrous MeOH (4 mL, 0.92 M) over 5 minutes. The reaction was monitored by TLC analysis (hexane/EtOAc 4:1, CAM) until consumption of starting material was observed (15 minutes). The reaction mixture turned from an initial clear and colorless solution to a clear and gold color after addition of NaOMe. The reaction mixture was concentrated to dryness and reconstituted in EtOAc. The crude organic mixture was washed with small portions of H_2O (2 x 5 mL) and the organic phase was subsequently dried over anhydrous Na_2SO_4, filtered, and concentrated to dryness to give 761 mg of crude product as a gold oil. The crude material was chromatographed on silica gel (5:1 → 4:1 hexanes/EtOAc) to yield 483 mg (58% yield) of epoxy methyl ester **395** as a soft white solid.

395: R_f = 0.37 (4:1 hexanes/EtOAc); mp = 65 - 66 °C ($CHCl_3$); $[\alpha]_D^{20}$ = -130.2 (*c* 1.0, $CHCl_3$); FT-IR (film) ν 2992, 2950, 1742, 1435, 1371, 1253, 1209, 1097, 1061, 1042, 858, 776 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 6.16 (ddd, *J* = 1.71, 0.01, 10.15 Hz, 1 H), 5.97 (ddd, *J* = 1.13, 2.59, 10.15 Hz, 1H), 5.01 (t, *J* = 2.15 Hz, 1H), 3.91 (s, 3H), 3.71 (d, *J* = 3.78 Hz, 1H), 3.45 (dt, *J* = 1.07, 1.88 Hz, 1H), 1.43 (s, 3H), 1.41 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 171.7, 132.9, 123.5, 113.00, 80.4, 72.3, 53.2, 51.5, 47.1, 28.2, 27.2; MS (EI+) *m/z* (%) 211(36), 169 (9), 167 (100), 137 (24), 109 (64), 81 (75), 59 (17), 53 (33), 43 (87), 41 (8); HRMS calcd. for $C_{11}H_{14}O_5$: 226.0841; Found: 227.0919 (M+1). Anal calcd.: C 58.40, H 6.24. Found: C 58.14, H 6.22.



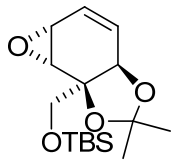
406

[(*3aR,5aR,6aR,6bR*)-2,2-Dimethyl-5a,6a-dihydrooxireno[e][1,3]benzodioxol-6b(3aH)-yl]methanol (406).

To a chilled solution (ice-bath) of epoxy ester **395** (634 mg, 2.80 mmol) in anhydrous MeOH (14 mL) was added $NaBH_4$ (1.06 g, 28.0 mmol) with stirring. The reaction was kept at ~5 °C (ice-bath) and monitored by TLC analysis for 2 hours prior to quenching with NH_4Cl (saturated solution, 2.5 mL) at 0 °C. The quenched reaction mixture was allowed to warm to room temperature with stirring and the resultant white slurry was filtered and washed with EtOAc. The filtrate was concentrated *in vacuo* and extracted with EtOAc (5 x 4mL), the organic phases were combined, dried over anhydrous Na_2SO_4, filtered and concentrated to yield a light brown oil with a mass of 503 mg. The crude

material was subjected to silica gel chromatography (1:1 hexanes/EtOAc) to yield 360 mg (65%) of alcohol **406** as a white solid.

406: R_f = 0.45 (hexanes/EtOAc 1:1, CAM); mp = 61 – 63 °C ($CHCl_3$); $[\alpha]_D^{20}$ = 69.6 (*c* 0.95, $CHCl_3$); FT-IR (film) ν 3480, 2991, 2928, 1370, 1240, 1217, 1160, 1055, 998, 896, 836 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 6.19 (ddd, *J* = 1.45, 3.79, 10.09 Hz, 1H), 6.00 (ddd, *J* = 1.03, 3.02, 10.11 Hz, 1H), 4.24 (dd, *J* = 1.44, 3.03 Hz, 1H), 3.84 (m, 2H), 3.62 (d, *J* = 3.75 Hz, 1H), 3.45 (td, *J* = 0.93, 3.75 Hz, 1H), 2.12 (dd, *J* = 4.65, 8.55 Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 132.8, 124.8, 111.7, 79.5, 72.6, 66.6, 51.9, 47.4, 28.7, 28.5; MS (EI+) *m/z* (%) 199 (12), 183 (11), 167 (19), 165 (14), 155 (33), 153 (15), 152 (21), 149 (10), 141 (39), 139 (18), 138 (41), 137 (28), 135 (14), 129 (10), 128 (10), 127 (11), 125 (14), 124 (19), 123 (39), 121 (22), 120 (25), 119 (12), 115 (16), 113 (15), 111 (40), 110 (11), 109 (36), 108 (19), 107 (61), 106 (15), 105 (32), 97 (21), 95 (46), 93 (22), 90 (40), 89 (63), 81 (46), 79 (26), 71 (30), 69 (66), 65 (28), 59 (42), 55 (67), 43 (100), 41 (51), 39 (36), 29 (19); HRMS calcd. for $C_9H_{11}O_4$ (M-CH_3): 183.0657, Found : 183.0657; Anal cald.: C 60.59, H 7.12. Found: C 60.38, H 7.17.

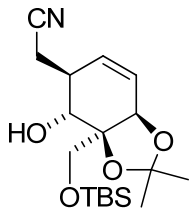


408

***tert*-Butyl (((*3aR,5aR,6aR,6bR*)-2,2-dimethyl-5a,6a-dihydrooxireno[2',3':3,4]benzo[1,2-*d*][1,3]dioxol-6b(3a*H*) yl)methoxy)dimethylsilane (408)**.

To a solution of alcohol **406** (700 mg, 3.53 mmol) in anhydrous DMF (6.6 mL) was added imidazole (312 mg, 4.59 mmol) with stirring. To this mixture was added *t*-butyldimethylsilyl chloride (691 mg, 4.59 mmol) and the reaction was monitored by TLC analysis (hexanes/EtOAc 1:1, CAM) and deemed complete after 45 minutes. The reaction mixture was diluted with water (30 mL) and allowed to stir for several minutes. The aqueous phase was extracted with Et_2O (4 x 7 mL), the organic phases were combined, dried over $MgSO_4$, filtered and concentrated to yield 1.05 g (95%) of TBS-protected epoxide **408** as a colorless oil that needed no further purification. An analytical sample was prepared by passing through a plug of silica gel.

408: R_f = 0.87 (hexanes/EtOAc 1:1); $[\alpha]_D^{20}$ = -54.9 (*c* 0.95, $CHCl_3$); FT-IR (film) 3685, 3020, 2400, 2253, 1794, 1523, 1474, 1425, 1382, 1212, 1097, 907, 780 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 6.16 (ddd, *J* = 1.47, 3.81, 10.08 Hz, 1H), 5.94 (ddd, *J* = 1.10, 2.94, 10.08 Hz, 1H), 4.20 (dd, *J* = 1.50, 2.88 Hz, 1H), 3.84 (dd, *J* = 10.52, 23.88 Hz, 2H), 3.58 (d, *J* = 3.78 Hz, 1H), 3.40 (dt, *J* = 0.98, 1.89 Hz, 1H), 1.48 (s, 3H), 1.38 (s, 3H), 0.95 (s, 9H), 0.13 (s, 6H); ^{13}C ($CDCl_3$, 75 MHz) δ 132.9, 124.7, 111.6, 80.3, 77.2, 77.0, 76.8, 72.5, 66.9, 51.7, 47.1, 28.8, 28.7, 25.9, 18.5, -5.3, -5.4; MS (FAB) *m/z* (%) 313 (7), 297 (10), 255 (38), 239 (11) 209 (24), 198 (13), 197 (60), 181 (12), 179 (11), 169 (55), 167 (83), 151 (54), 139 (20), 129 (20), 123 (68), 115 (43), 109 (24), 89 (70), 81 (70), 73 (100), 59 (68), 43 (60), 29 (20); HRMS calcd. for $C_{16}H_{28}O_4Si$: 312.1757, Found: 313.1835; Anal calcd.: C 61.50, H 9.03. Found: C 61.48, H 8.99.



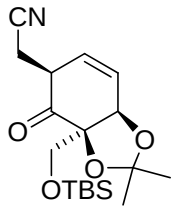
409

2-((*3aS,4R,5S,7aR*)-3a-((tert-Butyldimethylsilyoxy)methyl)-4-hydroxy-2,2-dimethyl-3a,4,5,7a-tetrahydrobenzo[*d*][1,3]dioxol-5-yl)acetonitrile (409).

To a solution of *n*BuLi (2.3 M in hexanes) in THF (11 mL) at -72 °C (external temperature) was added anhydrous acetonitrile (0.93 mL) over 1 minute with vigorous stirring; the clear and colorless mixture slowly turned to a slightly off-white (yellow) slurry within a minute of addition of acetonitrile. The reaction mixture was allowed to stir at < -65 °C for 35 minutes prior to cooling the mixture to -83 °C and performing the addition of epoxide **408** (930 mg, 2.97 mmol) in anhydrous THF (10 mL) over 10 minutes (temperature after addition was -75 °C). The reaction was allowed to warm to -55 °C at which point the reaction mixture became homogeneous and orange in color. The reaction vessel was moved to an ice-bath and the reaction was monitored by TLC analysis (hexanes/EtOAc 5:1, CAM). Starting material **408** was consumed approximately 20 minutes after warming to 0 °C. The reaction mixture was quenched with NH_4Cl (sat'd aqueous solution, 1.5 mL) at 0 °C at which point the mixture turned from dark orange to light yellow color. Water (6 mL) was added and the aqueous mixture was stirred at 0 °C for several minutes, warming to room temperature. The phases were allowed to separate and the aqueous layer (pH 8) extracted with EtOAc (3x). The organic phases were combined, dried over Na_2SO_4, filtered and concentrated to yield 1.37 g of an orange oil.

The crude mixture was purified by silica gel chromatography (hexanes/EtOAc 5:1 → 3:1) and provided 840 mg (80%) alcohol **409** as a very viscous, colorless oil.

409: R_f = 0.31 (3:1 hexanes/EtOAc); $[\alpha]_D^{20}$ = -54.9 (*c* 1, $CHCl_3$); FT-IR (film) ν 3437, 2930, 2857, 2246, 1471, 1381, 1253, 1216, 1158, 1095, 1055, 839 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 5.99 (ddd, *J* = 2.52, 3.95, 9.96 Hz, 1H), 5.89 (d, *J* = 10.17 Hz, 1H), 4.51 (d, *J* = 3.87 Hz, 1H), 3.85 (dd, *J* = 10.97, 16.22 Hz, 2H), 3.63 (dd, *J* = 4.38, 10.11 Hz, 1H), 3.08 (d, *J* = 4.53 Hz, 1H), 2.87 (dd, *J* = 4.11, 16.57 Hz, 1H), 2.64 (m, *J* = 3.40 Hz, 1H), 2.41 (dd, *J* = 9.00, 16.57 Hz, 1H), 1.52 (s, 3H), 1.44 (s, 3H), 0.90 (s, 9H), 0.10 (s, 3H), 0.09 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 131.3, 125.5, 118.2, 110.1, 82.5, 75.1, 74.3, 62.8, 38.7, 28.7, 26.5, 25.8, 20.0, 18.2, -5.6, -5.7; MS (EI+) *m/z* (%) 338 (6), 238 (24), 220 (16), 209 (13), 208 (73), 191 (11), 190 (61), 167 (18), 105 (16), 89 (21), 75 (100), 73 (46), 59 (42), 43 (27), 41 (10); HRMS calcd. for $C_{17}H_{28}O_4NSi$ (M-CH_3): 338.1788, Found: 338.17796; Anal calcd.: C 61.15, H 8.84. Found C 61.26, H 8.82.



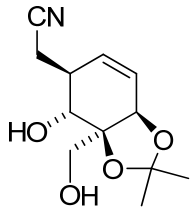
444

2-((*3aR,5S,7aR*)-3a-((tert-Butyldimethysilyloxy)methyl)-2,2-dimethyl-4-oxo-3a,4,5,7a-tetrahydrobenzo[*d*][1,3]dioxol-5-yl)acetonitrile (444).

A solution of alcohol **409** (12 mg, 0.033 mmol) in methylene chloride (0.5 mL) was chilled in an ice-bath and DMP (28 mg, 0.067 mmol) was added as one portion. The reaction mixture was removed from the ice-bath after 15 minutes and allowed to warm to room temperature. The reaction was monitored by TLC analysis (hexanes/EtOAc 3:1,

CAM) and starting material was consumed after an additional portion of DMP (7 mg, 0.015 mmol) was added and stirred overnight. The reaction mixture appeared as a white slurry and diluted with Et_2O (1 mL), filtered, and the filtered solid washed with Et_2O (1 x 1 mL, 1 x 0.5 mL). The filtrate was washed with $NaHCO_3$ (10% aqueous solution, 2 x 0.2 mL), water (1 x 0.2 mL), and brine (1 x 0.2 mL). The organics were dried over $MgSO_4$, filtered, and concentrated to provide 9 mg (81%) of a white crystalline solid, identified as ketone **444**.

444: R_f = 0.44 (hexanes/EtOAc 3:1); $[\alpha]_D^{20}$ = -63.5 (*c* 0.3, $CHCl_3$); IR (film) ν 2930, 2857, 2247, 1734, 1470, 1380, 1254, 1218, 1097, 838, 780 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.14 (td, *J* = 3.5, 10.0 Hz, 1H), 5.99 (dd, *J* = 2.1, 10.0 Hz, 1H), 4.82 (dd, *J* = 1.3, 3.8 Hz, 1H), 3.89 (d, *J* = 10.5 Hz, 1H), 3.80 (d, *J* = 10.2 Hz, 1H), 3.56 (m, *J* = 3.1 Hz, 1H), 2.90 (dd, *J* = 5.2, 17.3 Hz, 1H), 2.53 (dd, *J* = 8.9, 17.1 Hz, 1H), 1.40 (s, 6H), 0.87 (s, 9H), 0.08 (s, 3H), 0.06 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 204.1, 129.2, 127.3, 117.8, 110.9, 85.9, 78.9, 66.4, 44.8, 27.4, 26.5, 25.7, 18.1, 17.4, -5.6; MS (+EI) *m/z* (%) 336 (4), 248 (15), 236 (51), 208 (38), 207 (18), 206 (100), 204 (11), 190 (15), 179 (24), 178 (29), 167 (14), 166 (38), 151 (18), 120 (54), 115 (11), 89 (36), 77 (11), 75 (85), 73 (71), 65 (10), 59 (23), 58 (18), 57 (17), 45 (15), 43 (64), 41 (19); HRMS (+EI) calcd for $C_{18}H_{29}NO_4Si$: 336.1625. Found 336.1631.

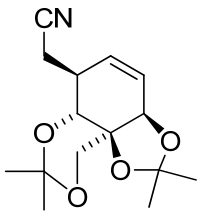


410

2-((*3aS,4R,5S,7aR*)-4-Hydroxy-3a-(hydroxymethyl)-2,2-dimethyl-3a,4,5,7a-tetrahydrobenzo[d][1,3]dioxol-5-yl)acetonitrile (410).

To a solution of TBS-protected alcohol **409** (466 mg, 1.31 mmol) in THF (13 mL) at room temperature was added a 1.0 M solution of tetrabutylammonium fluoride in THF (3.95 mL, 3.95 mmol) with stirring. The clear and colorless mixture turned to a clear and light brown color upon addition of TBAF and the reaction was monitored by TLC analysis (EtOAc). After 30 minutes the reaction mixture was quenched at room temperature with a saturated solution of NH_4Cl (1 mL) and concentrated *in vacuo*. The concentrated reaction mixture was diluted with H_2O and extracted with EtOAc (3x). The organic phases were combined, concentrated, passed through a plug of silica gel and eluted with EtOAc. The organic phase was dried over $MgSO_4$ and concentrated to yield 226 mg (72%) of diol **410** as a low melting white solid that required no further purification.

410: R_f = 0.43 (EtOAc); $[\alpha]_D^{20}$ = -27.9 (*c* 1.0, $CHCl_3$); FT-IR (film) ν 3343, 3219, 2983, 2890, 2236, 1426, 1366, 1220, 1164, 1072, 1047, 1034, 913, 866, 768, 744, 663 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 6.02 (ddd, *J* = 2.4, 4.2, 10.0 Hz, 1H), 5.92 (d, *J* = 10.0 Hz, 1H), 4.54 (d, *J* = 4.2 Hz, 1H), 4.06 (dd, *J* = 3.2, 12.1 Hz, 1H), 3.74 (dd, *J* = 3.8, 9.9 Hz, 1H), 3.56 (dd, *J* = 9.0, 12.0 Hz, 1H), 2.87 (dd, *J* = 3.4, 15.9 Hz, 1H), 2.68 (d, *J* = 4.4 Hz, 1H), 2.49 (m, *J* = 9.6 Hz, 2H), 2.03 (dd, *J* = 3.6, 9.1 Hz, 1H), 1.56 (s, 3H), 1.48 (s, 3H) ; ^{13}C NMR ($CDCl_3$, 150 MHz) δ 131.3, 125.2, 110.4, 83.0, 74.4, 73.7, 60.1, 41.5, 38.2, 28.9, 26.6, 20.0; MS (+EI) *m/z* (%) 338 (9), 323 (18), 238 (27), 221 (10), 209 (20), 208 (85), 193 (16), 169 (22), 105 (13), 89 (34), 75 (100), 73 (55), 59 (48), 41 (10); HRMS calcd for $C_{11}H_{14}O_4N$ (M-CH_3) 224.0923, Found: 224.0964.

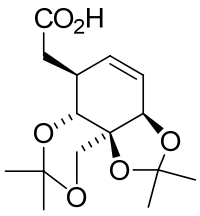


411

2-((*4aR7aR,10S,10aR*)-2,2,6,6-Tetramethyl-10,10a-dihydro-4*H*,7a*H*-[1,3]dioxolo[4'5':2,3]benzo[1,2-*d*][1,3]dioxin-10-yl)acetonitrile (411).

To a mixture of diol **410** (220 mg, 0.835 mmol) and 2,2-dimethoxypropane (1.02 mL, 8.35 mmol) in acetone (8.3 mL) at room temperature was added a catalytic amount of *p*-toluenesulfonic acid monohydrate (15 mg) with stirring. The reaction was monitored by TLC analysis (hexanes/EtOAc 1:1) and **410** was observed to be consumed after 95 minutes. The reaction mixture was quenched with a 10% aqueous solution of $NaHCO_3$ (2 mL), concentrated and subsequently re-diluted with EtOAc and H_2O. The phases were separated and the aqueous phase extracted with EtOAc (2x). The combined organic phases were dried over $MgSO_4$ and concentrated to provide 229 mg (98%) of a light brown oil as bisacetonide **411** that was used without further purification and prolonged storage.

411: R_f = 0.73 (hexanes/EtOAc 1:1); $[\alpha]_D^{18}$ = 18.0 (*c* 2.4, $CHCl_3$); FT-IR (film) ν 2993, 2938, 2878, 2248, 1595, 1383, 1371, 1236, 1076, 1043, 857, 665 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 5.92 (d, *J* = 10.0 Hz, 1H), 5.83 (dd, *J* = 4.4, 10.3 Hz, 1H), 4.80 (s, 1H), 4.23 (s, 1H), 3.82 (d, *J* = 11.2 Hz, 1H), 3.75 (d, *J* = 11.3 Hz, 1H), 2.76 (dd, *J* = 8.7, 16.6 Hz, 1H), 2.63 (dd, *J* = 7.8, 16.5 Hz, 1H), 2.55 (q, *J* = 6.6 Hz, 1H), 1.56 (s, 3H), 1.40 (s, 3H), 1.39 (s, 3H), 1.38 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 128.3, 126.2, 118.3, 110.7, 99.0, 75.2, 73.5, 69.5, 65.7, 37.0, 30.3, 29.1, 28.5, 21.0, 18.5.



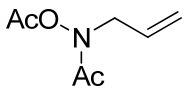
412

2-((*4aR7aR,10S,10aR*)-2,2,6,6-Tetramethyl-10,10a-dihydro-4*H*,7a*H*-[1,3]dioxolo[4'5':2,3]benzo[1,2-*d*][1,3]dioxin-10-yl)acetic acid (412).

A white slurry of crude nitrile **411** (191 mg, 0.683 mmol) in a mixture of aqueous NaOH (15% *w/w*, 4.5 mL) and MeOH (2.7 mL) was heated to reflux for 40 minutes while monitored by TLC analysis (hexanes/EtOAc 1:1). Upon consumption of **411** the reaction was cooled to 0 °C in an ice-bath and neutralized with 6 M HCl. The neutral mixture was extracted with DCM (4x) and EtOAc (2x), the combined organic phases dried over Na_2SO_4 and concentrated to yield 197 mg (97%) of an off-white, foam-like solid **412**. The material required no further purification. An analytical sample was acquired by silica gel chromatography (EtOAc) as a sticky (hygroscopic) white solid.

412: R_f = 0.61 (streak, EtOAc); $[\alpha]_D^{20}$ = -92.0 (*c* 1, $CHCl_3$); IR (film) ν 3192, 2993, 2938, 1733, 1709, 1382, 1289, 1236, 1160, 1076, 920, 858, 734, 519 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 10.85 (s, 1H), 5.78 (m, 2H), 4.76 (s, 1H), 4.19 (s, 1H), 3.78 (d, *J* = 11.16 Hz, 1H), 3.71 (d, *J* = 11.19 Hz, 1H) 2.80 (dd, *J* = 7.59, 15.76 Hz, 1H), 2.62 – 2.68 (m, 1H), 2.56 (dd, *J* = 6.62, 15.71 Hz, 1H) 1.51 (s, 3H), 1.40 (s, 3H), 1.37 (s, 3H), 1.35 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 177.8, 128.3, 126.4, 110.5, 98.8, 75.4, 73.6, 70.2, 65.8, 36.8, 36.3, 30.3, 29.0, 28.4, 18.6; MS (+EI) *m/z* (%) 283 (36), 282 (26), 225 (19), 223 (11), 182 (26), 181 (20), 169 (23), 168 (12), 165 (52), 164 (79), 163 (13), 152 (100),

151 (27), 137 (25), 123 (26), 120 (21), 108 (84), 107 (69), 95 (28), 91 (32), 83 (17), 81 (22), 79 (46), 77 (37), 65 (15), 55 (12), 53 (13), 43 (97); HRMS (+EI) calcd for $C_{14}H_{19}O_6$: 283.1176. Found 283.1181.

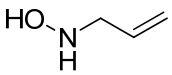


416

***N*-Acetoxy-*N*-allylacetamide (416)**.

To a solution of **415** (2.0 g, 17.0 mmol) in anhydrous DMF (80 mL) at room temperature was added K_2CO_3 (2.1 g, 15.4 mmol, 1.1 equiv) with stirring. To this mixture was added allyl bromide (2.2 mL, 25.5 mmol, 1.5 equiv) as a single portion and the reaction was monitored by TLC analysis (hexanes/EtOAc 1:1). Upon consumption of **415** the reaction mixture was filtered and the DMF was distilled under reduced pressure. The crude material was purified by silica gel chromatography (hexanes/EtOAc 1:1) and yielded 1.20 g (46%) of **416** as a light brown, non-viscous oil whose spectral data matched literature values.[270, 282]

416: ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.90 – 5.77 (m, 1H), 5.30 – 5.22 (m, 2H), 4.32 (d, J = 6.1 Hz, 2H), 2.20 (s, 3H), 2.06 (s, 3H).[270, 282]

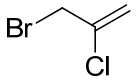


413

***N*-allylhydroxylamine (413)**. A solution of **416** (2.26 g, 14.3 mmol) in 3 M aqueous HCl (32 mL) was heated to reflux and monitored by TLC analysis (hexanes/EtOAc 1:1) until the consumption of **416** was observed (*ca* 1.5 h). The reaction mixture (clear and

colorless) was made alkaline with 6 M aqueous NaOH and extracted with Et_2O (5x). The organic phase was dried over $MgSO_4$, filtered and concentrated to yield 0.64 g (61%) of **413** as colorless needle-like crystals.[282]

413: ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.94 (tdd, *J* = 6.4, 10.4 Hz, 17.2, 1H), 5.91 (td, *J* = 6.4, 10.2 Hz, 1H), 5.32 – 5.20 (m, 2H), 3.59 (t, *J* = 1.1 Hz, 1H), 3.57 (t, *J* = 1.3 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 133.8, 118.5, 56.6.[282]

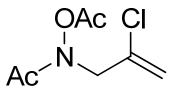


432

3-bromo-2-chloroprop-1-ene (432).

To a slurry of NaBr (11.6 g, 112 mmol) in anhydrous DMF (100 mL) at room temperature was added 2,3-dichloropropene (4.1 mL, 45 mmol) with stirring. The reaction mixture was heated to 90 ˚C at which point the reaction mixture turned into a thick white slurry. After stirring at 90 ˚C for 16 h the reaction mixture was quenched with H_2O (50 mL). The aqueous phase was extracted with pentanes (3 x 100 mL) and the organic phases were collected ad concentrated under reduced pressure at 0 – 5 ˚C. The crude material was distilled under reduced pressure to obtain 1.02 g (15%) of **432** as a colorless liquid that was used without further purification.

432: ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.61 (s, 1H), 5.42 (d, *J* = 1.9 Hz, 1H), 4.11 (s, 2H).

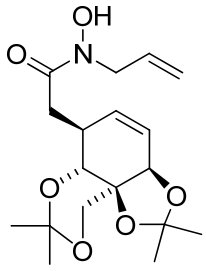


433

***N*-Acetoxy-*N*-(2-chloroallyl)acetamide (433)**.

To a reaction slurry that contained K_2CO_3 (0.91 g, 6.62 mmol), *N*,*O*-bisacetate **415** (0.62 g, 5.30 mmol), and DMF (10 mL) was added the freshly prepared vinyl chloride **432** (0.99 g, 6.36 mmol) with stirring and at room temperature. Upon consumption of **415**, as indicated by TLC (hexanes/EtOAc 1:1), the reaction mixture was filtered the DMF was distilled under reduced pressure. The remaining mother liquor was chromatographed on silica gel (hexanes/EtOAc 1:1) and afforded 0.42 g (35%) of **433** as a colorless oil that corresponded with the values reported in the literature. The remaining material was present as contaminated material in other chromatography fractions.[8]

433: ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.42 (d, *J* = 2.7 Hz, 2H), 4.48 (bs, 2H), 2.22 (s, 3H), 2.09 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 168.2, 135.4, 118.2, 115.7, 115.6, 20.1, 18.3.[8]

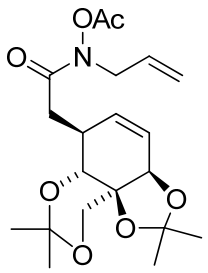


419

***N*-Allyl-*N*-hydroxy-2-((*4aR,7aR,10S,10aR*)-2,2,6,6-tetramethyl-10,10a-dihydro-4*H*,7a*H*-[1,3]dioxolo[4',5':2,3]benzo[1,2-*d*][1,3]dioxin-10yl)acetamide (419)**.

To a chilled solution of carboxylic acid **412** (254 mg, 0.851 mmol) in THF (8.5 mL) was added triethylamine (130 μL, 0.936 mmol) followed by isobutyl chloroformate (122 μL, 0.936 mmol). The clear and colorless solution immediately turned to a white slurry upon addition of isobutyl chloroformate. The reaction mixture was stirred at 0 °C until consumption of **412** was observed by TLC (hexanes/EtOAc 1:1) (10 minutes). A solution of *N*-allylhydroxylamine **413** (62 mg, 0.851) in THF (0.5 mL) was added to the cooled reaction mixture and the reaction was monitored by TLC (hexanes/EtOAc 1:1). After 5 minutes the reaction mixture was filtered and the filtered solid was washed with EtOAc. The filtrate was concentrated *in vacuo* to provide the crude material as a colorless oil (350 mg). The crude material was chromatographed on silica gel (hexanes/EtOAc 1:1) to yield **419** (170 mg, 56%) as a colorless oil.

419: R_f = 0.34 (hexanes/EtOAc 1:1); $[\alpha]_D^{20}$ = +25.3 (*c* 0.45, $CHCl_3$); IR (film) υ 3685, 3020, 2430, 2400, 1602, 1521, 1476, 1423, 1384, 1208, 1075, 1015, 928, 850, 731, 669 cm^{-1}; ^1H NMR (600 MHz, DMSO) δ 9.71 (s, 1H), 5.84 – 5.74 (m, 1H), 5.72 (dd, *J* = 4.5, 10.3 Hz, 1H), 5.66 (dd, *J* = 1.7, 10.3 Hz, 1H), 5.23 – 5.13 (m, 1H), 4.59 – 4.55 (m, 1H), 4.19 – 4.06 (m, 2H), 3.63 (s, 1H), 2.77 (dd, *J* = 7.6, 16.7 Hz, 1H), 2.62 (dd, *J* = 7.0, 16.5 Hz, 1H), 2.56 – 2.52 (m, 1H), 1.43 (s, 3H), 1.34 (s, 3H), 1.30 (s, 3H), 1.24 (s, 3H); ^{13}C NMR (150 MHz, DMSO) δ 171.9, 133.4, 130.4, 125.7, 117.8, 110.1, 98.5, 79.7, 75.6, 73.5, 70.6, 65.8, 50.8, 36.2, 35.1, 30.6, 29.4, 28.8, 19.1; MS (+EI) *m/z* (%) 352 (1), 338 (15), 322 (22), 295 (18), 220 (41), 204 (28), 164 (28), 147 (66), 135 (28), 107 (59), 56 (48), 43 (97), 41 (100); HRMS (+EI) calcd for $C_{17}H_{24}O_6N$: 338.1598. Found 338.1604.



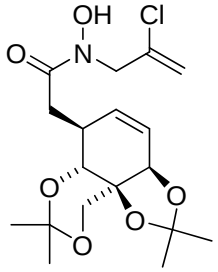
385

***N*-Acetoxy-*N*-allyl-2-((*4aR,7aR,10S,10aR*)-2,2,6,6-tetramethyl-10,10a-dihydro-4*H*,7a*H*-[1,3]dioxolo[4',5':2,3]benzo[1,2-*d*][1,3]dioxin-10yl)acetamide (385)**.

To a chilled (ice-bath) solution of carboxylic acid **397** (27 mg, 0.090 mmol) in THF (0.9 mL) was added Et_3N (12 μL, 0.090 mmol) with stirring. The mixture was allowed to stir for 10 minutes prior to addition of isobutyl chloroformate (13 μL, 0.099 mmol); the reaction mixture turned from clear and colorless to a hazy-white slurry. After 15 minutes another portion of Et_3N (24 μL, 0.180 mmol) was added followed by freshly prepared *N*-allyl-*O*-acetyl hydroxylamine **417** (exess) in THF (0.2 mL) dropwise over 1 minute. The evolution of gas (CO_2, isobutylene) was observed and the reaction mixture remained as a white slurry. The reaction was monitored by TLC analysis (hexanes/EtOAc 3:1) and was allowed to warm to room temperature over several hours while stirred in an ice-bath. After 18.5 hours the reaction mixture (white slurry) was filtered and the filtered solid was washed with Et_2O. The filtrate was concentrated to dryness and the crude material was chromatographed as a complex mixture on silica gel (hexanes/EtOAc 2:1) to yield 2 mg (6%) of analytically pure **385** (more material available as impure fractions).

385: R_f = 0.27 (hexanes/EtOAc 3:1); $[\alpha]_D^{20}$ = +49.3 (*c* 0.3, $CHCl_3$); IR (film) ν 2992, 2934, 2873, 1795, 1676, 1432, 1382, 1366, 1236, 1177, 1075 cm^{-1}; ^1H NMR ($CDCl_3$, 600

MHz) δ 5.87-5.78 (m, 3H), 5.28-5.24 (m, 2H), 4.78 (s, 1H), 4.38-4.41 (m, 1H rotameric), 4.25-4.31 (m, 1H, rotameric), 4.16 (s, 1H), 3.78 (d, *J* = 11.25 Hz, 1H), 3.71 (d, *J* = 11.25 Hz, 1H), 2.80-2.77 (m, 2H), 2.40 (bs, 1H), 2.18 (s, 3H), 1.54 (s, 3H), 1.39 (s, 6H), 1.37 (s, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 171.6, 167.2, 131.3, 129.2, 126.2, 110.2, 98.8, 75.6, 73.7, 70.1, 65.9, 34.9, 30.4, 29.1, 28.5, 18.6, 18.4; MS (+EI) *m/z* (%) 380 (13), 261 (14), 223 (14), 165 (12), 164 (14), 147 (38), 137 (12), 136 (10), 135 (14), 134 (16), 120 (16), 119 (22), 113 (13), 108 (14), 107 (33), 95 (12), 91 (23), 85 (14), 81 (18), 77 (18), 71 (16), 69 (13), 59 (22), 56 (29), 55 (26), 43 (100), 42 (18), 41 (64); HRMS (+EI) calcd for $C_{20}H_{29}NO_7$: 395.1938. Found 395.1944.



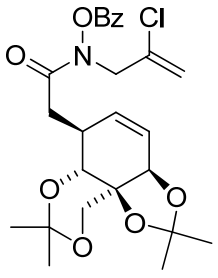
434

***N*-(2-Chloroallyl)-*N*-hydroxy-2-((*4aR,7aR,10S,10aR*)-2,2,6,6-tetramethyl-10,10a-dihydro-4*H*,7a*H*-[1,3]dioxolo[4',5':2,3]benzo[1,2-*d*][1,3]dioxin-10-yl)acetamide (434)**.

To a clear and colorless solution of carboxylic acid **412** (1.42 g, 4.75 mmol) in THF (30 mL) at 0 °C was added Et_3N (729 μL, 5.23 mmol) followed by isobutyl chloroformate (684 μL, 5.23 mmol); upon addition of isobutyl chloroformate the reaction mixture became a white slurry. The reaction was monitored by TLC (hexanes/EtOAc 1:1) and the consumption of **412** was observed after 10 minutes. The reaction mixture was maintained

at 0 °C and *N*-(2-chloroallyl)hydroxylamine **430** (904 mg, 5.94 mmol) in THF (17 mL) was added over the course of 3 minutes; no appearance change or exotherm was observable. The reaction was monitored by TLC (hexanes/EtOAC 1:1) and filtered after 45 minutes at 0 °C. The filtered solid was washed with EtOAc and the filtrate was concentrated *in vacuo* to provide an orange oil (2.74 g) that was chromatographed on silica gel (hexanes/EtOAc 1:1) to yield **434** (1.41 g, 69%) as a slightly off-white, hygroscopic foam after trituration with pentanes. The material decomposed upon standing and was used quickly after its preparation.

434: R_f = 0.34 (hexanes/EtOAc 1:1); mp = 49 °C (pentanes); $[\alpha]_D^{20}$ = +25.3 (*c* 0.45, $CHCl_3$); IR (film) υ 3192, 2993, 2938, 1733, 1709, 1382, 1289, 1236, 1160, 1076, 920, 858, 734, 519 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 9.88 (s, 1H), 5.74 (dd, *J* = 4.2, 10.4 Hz, 1H), 5.66 (dd, *J* = 2.3, 10.5 Hz, 1H), 5.44 (d, *J* = 14.8 Hz, 1H), 4.57 (bs, 1H), 4.38 (d, *J* = 16.1 Hz, 1H), 4.29 (d, *J* = 16.0 Hz, 1H), 4.13 (s, 1H), 3.63 (s, 2H), 2.82 (dd, *J*= 7.7, 16.4 Hz, 1H), 2.66 (dd, *J* = 7.2, 16.4 Hz, 1H), 2.56 (t, *J* = 5.5 Hz, 1H), 1.43 (s, 3H), 1.34 (s, 3H), 1.30 (s, 3H), 1.24 (s, 3H); ^{13}C NMR (75 MHz, DMSO) δ 170.7, 170.5, 170.1, 155.9, 109.6, 81.9, 81.8, 78.3, 73.5, 64.8, 62.2, 62.0, 52.8, 28.3, 27.2, 26.0, 21.2, 21.1, 20.9.

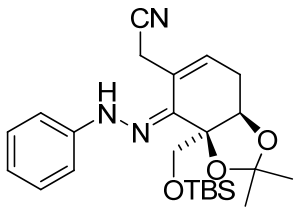


429

***N*-(Benzoyloxy)-*N*-(2-chloroallyl)-2-((*4aR,7aR,10S,10aR*)-2,2,6,6-tetramethyl-10,10a-dihydro-4*H*,7a*H*-[1,3]dioxolo[4',5':2,3]benzo[1,2-*d*][1,3]dioxin-10-yl)acetamide (429)**.

A solution of freshly prepared hydroxamic acid **434** (131 mg, 0.337 mmol) in methylene chloride (3.4 mL, 0.1 M) at 0 ˚C was added Et_3N (140 µL, 1.01 mmol, 3.0 equiv) followed by BzCl (117 µL, 1.01 mmol, 3.0 equiv) with stirring. The reaction was monitored by TLC analysis (hexanes/EtOAc 3:1) and **434** was consumed after 10 minutes. The reaction mixture was quenched with an aqueous NH_4Cl solution (sat'd, 0.3 mL) and H_2O (1 mL) at 0 ˚C. The phases were separated and the organic phase washed with H_2O (1x), brine (1x) and then dried over Na_2SO_4 prior to filtration and concentration *in vacuo* to yield 236 mg of crude material as an oily white preciptate. The material was chromatographed on silica gel (hexanes/EtOAc 3:1) and afforded 142 mg (86%) of **429** as a white foam-like solid (hygroscopic) after trituration with pentanes.

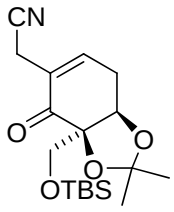
429: R_f = 0.40 (hexanes/EtOAc 3:1); $[\alpha]_D^{20}$ = +18.1 (*c* 0.40, $CHCl_3$); IR (liquid) ν 2994, 2939, 1633, 1456, 1412, 1423, 1384, 1288, 1234, 1161, 1075, 1049, 918, 850, 763, 739 cm^{-1}; ^1H NMR (300 MHz, DMSO) δ 8.07 – 7.87 (m, 1H), 7.67 (t, *J* = 7.5 Hz, 1H), 7.51 (t, *J* = 7.7 Hz, 1H), 5.65 (dd, *J* = 4.4, 10.3 Hz, 1H), 5.56 (dd, *J* = 1.8, 10.4 Hz, 1H), 5.51 (s, 1H), 5.37 (d, *J* = 1.8 Hz, 1H), 4.60 (d, *J* = 16.4 Hz, 1H), 4.53 (d, *J* = 16.4 Hz, 1H), 4.46 (bs, 1H), 4.03 (s, 1H), 3.53 (s, 2H), 2.91 (s, 1H), 2.78 (dd, *J* = 8.1, 15.9 Hz, 1H), 2.40 (dd, *J* = 6.4, 15.8, 1H), 1.36 (s, 3H), 1.16 (s, 3H), 1.05 (s, 3H), 0.98 (s, 3H); ^{13}C NMR (75 MHz, DMSO) δ 164.3, 135.8, 135.03, 130.0, 129.5, 127.1, 126.3, 117.0, 110.0, 98.7, 75.9, 73.6, 70.3, 66.1, 54.4, 36.7, 35.1, 30.2, 29.2, 28.4, 19.4; Anal calcd for $C_{25}H_{30}NO_7Cl$: C, 61.03; H, 6.15. Found: C, 60.94; H, 6.21.



477

2-((*3aR,7aR,E*)-3a-((tert-Butyldimethylsilyloxy)methyl)-2,2-dimethyl-4-(2-phenylhydrazono)-3a,4,7,7a-tetrahydrobenzo[*d*][1,3]dioxol-5-yl)acetonitrile (477).

To a solution of **444** (30 mg, 0.085 mmol) in glacial HOAc (0.5 mL) was added phenylhydrazine (21 μL, 0.213 mmol, 2.5 equiv) at room temperature and with stirring. The reaction was heated to 95 ˚C and monitored by TLC analysis (hexanes/EtOAc 3:1). Ketone **444** was consumed after 2.5 h and the reaction was neutralized with a 1 M aqueous solution of NaOH. The aqueous mixture was extracted with methylene chloride (3 x 1 mL) and the combined organic phases were dried over Na_2SO_4, filtered and concentrated to yield 59 mg of crude material. Silica gel chromatography (hexanes/EtOAc 3:1) provided 23 mg (62%) of hydrazone **477** as an orange oil and 5 mg (17%) of enone **448** as a colorless oil.

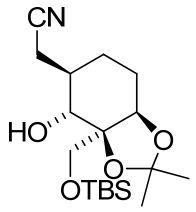


448

2-((*3aR,7aR*)-3a-((tert-Butyldimethylsilyloxy)methyl)-2,2-dimethyl-4-oxo-3a,4,7,7a-tetrahydrobenzo[*d*][1,3]dioxol-5-yl)acetonitrile (448): R_f = 0.24 (hexanes/EtOAc 3:1); FT-IR (film) ν 2987, 2954, 2929, 2857, 2250, 1734, 1679, 1471, 1383, 1373, 1253, 1093,

1006, 991, 840, 779 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.07 (m, 1H), 4.57 (m, 1H), 4.10 (d, *J* = 9.4 Hz, 1H), 3.68 (d, *J* = 9.4 Hz, 1H), 3.37 (m, 2H), 2.95 (m, 2H), 1.40 (s, 3H), 1.31 (s, 3H), 0.83 (s, 9H), 0.04 (s, 3H), -0.02 (s, 3H).

477: R_f = 0.57 (hexanes/EtOAc 3:1); FT-IR (film) ν 3318, 2928, 2850, 2247, 1737, 1603, 1563, 1505, 1471, 1372, 1258, 1237, 1093, 840, 752 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 9.61 (s, 1H), 7.32 (d, *J* = 7.6 Hz, 2H), 7.10 (d, *J* = 7.4 Hz, 2H), 6.92 (t, *J* = 7.2 Hz, 1H), 6.05 (m, *J* = 4.1 Hz, 1H), 4.44 (m, 1H), 4.04 (d, *J* = 10.7 Hz, 1H), 3.85 (d, *J* = 10.7 Hz, 1H), 3.52 (bs, 2H), 2.76 (m, 2H), 1.48 (s, 3H), 1.36 (s, 3H), 0.87 (s, 9H), 0.05 (s, 3H), 0.02 (s, 3H); MS (+EI) *m/z* (%) 443 (5), 442 (18), 441 (55), 326 (17), 238 (41), 234 (12), 207 (16), 150 (19), 117 (11), 115 (15), 105 (13), 89 (48), 77 (45), 73 (100), 71 (17), 65 (17), 59 (20), 57 (26), 43 (61), 41 (28); HRMS (+EI) calcd for $C_{24}H_{35}N_3O_3Si$: 441.2440. Found 441.2447.



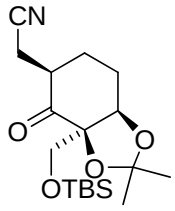
478

2-((*3aS,4R,5R,7aR*)-3a-((*tert*-Butyldimethylsilyloxy)methyl)-4-hydroxy-2,2-dimethylhexahydrobenzo[*d*][1,3]dioxol-5-yl)acetonitrile (478).

To a clear and colorless solution of alkene **409** (229 mg, 0.647 mmol) in absolute EtOH (6.5 mL, 0.1 M) at room temperature was added Pd/C (20 mg of a 10 wt% mixture, 10 *w*/*w*%) and the reaction vessel was purged with H_2 (1 atm). The reaction was monitored by TLC analysis (hexanes/EtOAc 3:1, CAM) and alkene **409** was consumed after 6 hours

and confirmed by crude NMR analysis. The reaction mixture was filtered through a pad of Celite and the filtrate concentrated *in vacuo* to yield a colorless oil, 213 mg (93%) **478**, that required no further purification. An analytical sample was obtained by silica gel chromatography (hexanes/EtOAc 3:1; 90% recovery).

478: R_f = 0.40 (hexanes/EtOAc 3:1, CAM); $[\alpha]_D^{18}$ = +89.5 (c = 1.0, $CHCl_3$); IR (film) *v* 3436, 2931, 2858, 1463, 1382, 1252, 1219, 1106, 1039, 839, 777, 665 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 4.30 (bs, 1H), 3.95 (d, *J* = 10.8 Hz, 1H), 3.74 (d, *J* = 10.8 Hz, 1H), 3.64 (d, *J* = 11.3 Hz, 1H), 2.69 (dd, *J* = 3.7, 16.8 Hz, 1H), 2.63 (bs, 1H), 2.40 (dd, *J* = 8.0, 16.7 Hz, 1H), 2.18 – 2. 16 (m, 1H), 2.02 – 2.00 (m, 1H), 1.82 – 1.76 (m, 2H), 1.67 – 1.63 (m, 1H), 1.53 (s, 3H), 1.39 (s, 3H), 0.92 (s, 9H), 0.11 (s, 3H), 0.10 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 118.6, 108.4, 83.3, 77.9, 63.1, 37.4, 28.5, 26.6, 25.9, 25.6, 25.1, 20.5, 18.3, -5.5, -5.6;MS (+EI) *m/z* (%) 340 (5), 241 (11), 240 (61), 222 (29), 211 (12), 210 (61), 192 (11), 182 (15), 181 (14), 117 (12), 105 (16), 75 (100), 73 (41), 59 (40), 43 (25), 41 (17); HRMS (+EI) calcd for $C_{17}H_{30}O_4NSi$: 340.1944. Found: 340.1935; Anal. Calcd for $C_{18}H_{33}O_4NSi$: C, 60.81; H, 9.36. Found C, 60.68; H, 9.41.

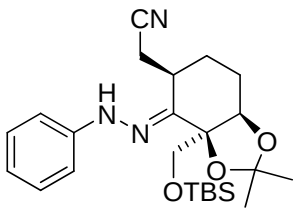


476

2-((*3aR,5R,7aR*)-3a-((*tert*-Butyldimethylsilyloxy)methyl)-2,2-dimethyl-4-oxohexahydrobenzo[*d*][1,3]dioxol-5-yl)acetonitrile (476).

To a clear and colorless solution of alcohol **478** (185 mg, 0.520 mmol) in methylene chloride (5.2 mL, 0.1 M) at room temperature was added commercial Dess-Martin periodinane (286 mg, 0.676 mmol, 1.3 equiv) in a single portion. The reaction mixture thickened to a white slurry within minutes of addition of DMP. The reaction was monitored by TLC analysis (hexanes/EtOAc 3:1, CAM) and another portion of DMP (44 mg, 0.104 mmol, 0.2 equiv) was added after 3 h. After an additional 50 minutes alcohol **478** was consumed based on TLC analysis and the reaction mixture was diluted with Et_2O (6 mL), filtered and the filtered solid rinsed with Et_2O (3 mL). The filtrate was concentrated *in vacuo* to yield 198 mg of crude material that was a slightly off-white solid. The material was chromatographed on silica gel (hexanes/EtOAc 3:1) to provide ketone **476** (113 mg, 62%) as a white solid.

476: mp 73 – 75 ˚C (Et_2O); R_f = 0..55 (hexanes/EtOAc 3:1, CAM); $[\alpha]_D^{20}$ = +45.3 (c = 0.3, $CHCl_3$); IR (film) *v* 2931, 2863, 1723, 1471, 1381, 1249, 1225, 1092, 839, 778, 665 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.41 (bs, 1H), 3.87 (d, *J* = 9.7 Hz, 1H), 3.67 (d, *J* = 9.7 Hz, 1H), 2.91 – 2.81 (m, 1H), 2.76 (dd, *J* = 4.7, 17.0 Hz, 1H), 2.46 (dd, *J* = 8.1, 17.0 Hz, 1H), 2.36 – 2.08 (m, 3H), 1.94 (dq, *J* = 3.6, 12.9 Hz, 1H), 1.36 (s, 3H), 1.34 (s, 3H), 0.89 (s, 9H), 0.07 (s, 3H), 0.04 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 209.1, 118.2, 108.8, 84.4, 80.9, 66.5, 47.4, 27.1, 26.2, 25.8, 25.8, 18.2, 17.5, -5.7, -5.8;MS (+EI) *m/z* (%) 338 (5), 238 (50), 210 (17), 209 (18), 208 (100), 206 (16), 180 (35), 167 (11), 143 (11), 89 (12), 75 (58), 73 (41), 59 (17), 43 (20), 41 (13); HRMS (+EI) calcd for $C_{17}H_{28}O_4NSi$: 338.1788. Found: 338.1788; Anal. Calcd for $C_{18}H_{31}O_4NSi$: C, 61.15; H, 8.84. Found C, 61.18; H, 8.88.



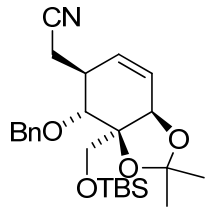
480

2-((*3aR,5R,7aR,E*)-3a-((*tert*-Butyldimethylsilyloxy)methyl)-2,2-dimethyl-4-(2-phenylhydrazono)hexahydrobenzo[*d*][1,3]dioxol-5-yl)acetonitrile (480).

To a solution of **476** (24 mg, 0.067 mmol) in pyridine (0.3 mL) was added phenylhydrazine hydrochloride (98 mg, 0.678 mmol, 10 equiv) and the reaction mixture was heated to reflux with stirring. The reaction was monitored by TLC analysis (hexanes/EtOAc 3:1) and upon consumption of **476** Celite was added to the reaction mixture and the mixture was concentrated *in vacuo*. The material was purified by silica gel column chromatography (hexanes/EtOAc 5:1 → 3:1) and **480** was isolated as an orange oil, 19 mg (66%).

480: R_f = 0.66 (hexanes/EtOAc 3:1); $[\alpha]_D^{18}$ = -172.2 (*c* 0.5, $CHCl_3$); FT-IR (film) ν 3320, 2928, 2850, 2245, 1604, 1560, 1500, 1471, 1373, 1258, 1240, 1094, 840, 752 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 9.84 (s, 1H), 7.26 (t, *J* = 7.8 Hz, 2H), 7.03 (d, *J* = 7.7 Hz, 2H), 6.85 (t, *J* = 7.3 Hz, 1H), 4.33 (bs, 1H), 3.97 (d, *J* = 10.5 Hz, 1H), 3.78 (d, *J* = 10.5 Hz, 1H), 2.95 (dd, *J* = 8.1, 19.6 Hz, 1H), 2.63 (dd, *J* = 7.3, 19.5 Hz, 2H), 2.27 (dd, *J* = 2.4, 13.8 Hz, 1H), 2.00 – 1.73 (m, 3H), 1.46 (s, 3H), 1.39 (s, 3H), 0.91 (s, 9H), 0.08 (s, 3H), 0.08 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 145.3, 140.0, 129.2, 119.7, 119.5, 112.6, 109.0, 84.3, 78.5, 63.2, 40.6, 26.8, 26.7, 26.0, 25.9, 25.5, 20.4, 18.3, -5.4, -5.6; MS (+EI) *m/z* (%) 444 (30), 443 (82), 329 (11), 238 (35), 298 (18), 241 (19), 240 (100), 236 (23), 235 (13), 212 (30), 195 (22), 150 (26), 149 (13), 115 (15), 105 (11), 93 (42), 55

(92), 89 (37), 77 (38), 75 (61), 74 (10), 73 (87), 65 (23), 59 (24), 57 (13), 43 (25), 41 (24); HRMS (+EI) calcd for $C_{24}H_{37}O_3N_3Si$: 443.2604. Found: 443.2592.



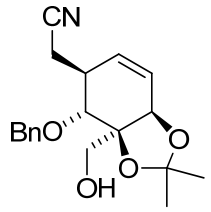
510

2-((*3aS,4R,5S,7aR*)-4-(Benzyloxy)-3a-((*tert*-butyldimethylsilyloxy)methyl)-2,2-dimethyl-3a,4,5,7a-tetrahydrobenzo[*d*][1,3]dioxol-5-yl) acetonitrile (510).

To a clear and colorless solution of alcohol **409** (139 mg, 0.393 mmol, 1.0 equiv) in THF at room temperature was added NaH (60% dispersed in mineral oil; 17 mg, 0.432 mmol, 1.1 equiv) as a single portion. Once the evolution of hydrogen ceased benzyl chloride (226 μL, 1.96 mmol, 5.0 equiv) and tetrabutylammonium chloride (145 mg, 0.393 mmol, 1.0 equiv) was added in succession and the reaction mixture was heated to reflux for ~24 h at which point TLC analysis (hexanes/EtOAc 3:1, CAM) indicated consumption of **409**. The reaction mixture was cooled to room temperature, concentrated *in vacuo*, and reconstituted in EtOAc. The white precipitate was filtered and the mother liquor (clear and yellow) was concentrated to a non-viscous and orange oil, 355 mg. The crude material was chromatographed on silica gel (hexanes/EtOAc 5:1 → 3:1) to yield 120 mg (69%) of **510** and 17 mg (10%) of **519** as colorless oils.

510: R_f = 0.68 (hexanes/EtOAc 3:1); $[\alpha]_D^{18}$ = +91.7 (c = 0.4, $CHCl_3$); IR (film) *v* 2951, 2926, 2855, 1469, 1456, 1378, 1365, 1251, 1213, 1160, 1084, 1054, 837, 776, 741, 665 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 7.37 (m, 5H), 6.03 – 6.00 (m, 1H), 5.85 (d, *J* = 10.9

Hz, 1H), 5.09 (d, J = 11.3 Hz, 1H), 4.72 (d, J = 3.8 Hz, 1H), 4.61 (d, J = 11.4 Hz, 1H), 3.92 (d, J = 10.9 Hz, 1H), 3.89 (d, J = 11.0 Hz, 1H), 3.54 (d, J = 10.3 Hz, 1H), 2.71 – 2.67 (m, 2H), 2.29 (dd, J = 9.7, 17.6 Hz, 1H), 1.61 (s, 3H), 1.49 (s, 3H), 0.91 (s, 9H), 0.10 (s, 3H), 0.09 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 138.2, 130.8, 128.6, 128.3, 128.0, 125.6, 118.2, 109.5, 84.4, 81.2, 74.6, 74.3, 61.6, 38.3, 28.7, 26.5, 25.9, 20.3, 18.4, -5.4, -5.6; MS (+EI) *m/z* (%) 428 (2), 328 (10), 190 (8), 92 (27), 91 (100), 89 (10), 75 (20), 73 (28), 57 (10), 43 (14); HRMS (+EI) calcd for $C_{24}H_{34}O_4NSi$: 428.2257. Found: 428.2261; Anal. Calcd for $C_{25}H_{37}O_4NSi$: C, 67.68; H, 8.41. Found C, 67.80; H, 8.35.



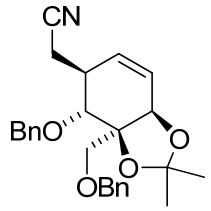
518

2-((*3aS,4R,5S,7aR*)-4-(Benzyloxy)-3a-(hydroxymethyl)-2,2-dimethyl-3a,4,5,7a-tetrahydrobenzo[*d*][1,3]dioxol-5-yl)acetonitrile (518).

To a clear and yellow solution of **510** (431 mg, 0.971 mmol) in THF (9.7 mL) at 5 ˚C was added a 1 M solution of tetrabutylammonium fluoride in THF (1.9 mL, 1.94 mmol, 2.0 equiv) over two minutes. The reaction was monitored by TLC analysis (hexanes/EtOAc 1:1) and **510** was consumed after 70 minutes. The reaction mixture was quenched with aqueous NH_4Cl (satd, 2 mL) at room temperature. The white precipitate was filtered from the reaction mixture and the mother liquor concentrated *in vacuo*. The aqueous phase mixture was extracted with EtOAc (2x) and the organic phase was washed with brine (1x), dried over Na_2SO_4, and concentrated *in vacuo* to 403 mg of crude material. The

crude material was chromatographed on silica gel (hexanes/EtOAc 1:1) to yield 310 mg (97%) of **518** as a colorless oil that required no further purification.

518: R_f = 0.50 (hexanes/EtOAc 1:1); $[\alpha]_D^{18}$ = +155.9 (*c* 0.4, $CHCl_3$); FT-IR (film) ν 3479, 3033, 2987, 2935, 2886, 2247, 1497, 1455, 1424, 1402, 1380, 1249, 1216, 1166, 1080, 1025, 910, 866, 813, 745, 699, 665 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 7.40 – 7.32 (m, 5H), 6.01 (td, *J* = 3.3, 9.8 Hz, 1H), 5.85 (d, *J* = 10.0 Hz, 1H), 5.10 (d, *J* = 11.2 Hz, 1H), 4.64 (d, *J* = 4.0 Hz, 1H), 4.61 (d, *J* = 11.2 Hz, 1H), 4.10 (d, *J* = 12.1 Hz, 1H), 3.64 (d, *J* = 9.8 Hz, 1H), 3.56 (d, *J* = 12.1 Hz, 1H), 2.67 (dd, *J* = 4.0, 16.7 Hz, 1H), 2.51 (t, *J* = 9.4 Hz, 1H), 2.36 (dd, *J* = 8.3, 16.7 Hz, 1H), 1.64 (s, 3H), 1.51 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 137.7, 130.8, 128.6, 128.2, 128.2, 125.4, 117.8, 109.9, 84.4, 80.8, 75.0, 73.8, 59.8, 38.1, 28.8, 26.7, 20.2; MS (+EI) *m/z* (%) 315 (1), 314 (3), 271 (3), 134 (7), 107 (32), 91 (100), 43 (11); HRMS (+EI) calcd for $C_{18}H_{20}O_4N$: 314.1396. Found: 314.1392; Anal. Calcd for $C_{19}H_{23}O_4N$: C, 69.28; H, 7.04. Found C, 69.41; H, 6.97.



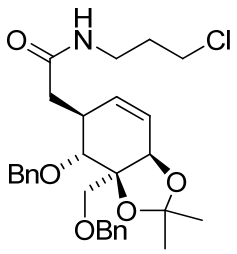
519

2-((*3aS,4R,5S,7aR*)-4-(Benzyloxy)-3a-((*tert*-butyldimethylsilyloxy)methyl)-2,2-dimethyl-3a,4,5,7a-tetrahydrobenzo[*d*][1,3]dioxol-5-yl) acetonitrile (519).

To a clear and colorless solution of alcohol **518** (31 mg, 0.094 mmol, 1.0 equiv) in THF at room temperature was added NaH (60% dispersed in mineral oil; 3 mg, 0.141 mmol, 1.5 equiv) as a single portion. Once the evolution of hydrogen ceased benzyl chloride (75

μL, 0.658 mmol, 7.0 equiv) and tetrabutylammonium iodide (35 mg, 0.0.94 mmol, 1.0 equiv) was added in succession and the reaction mixture was heated to 50 ˚C. The reaction was monitored by TLC analysis (hexanes/EtOAc 1:1, CAM) and additional Bu_4NI (7 mg, 0.018 mmol, 0.2 equiv) and BnCl (21 μL, 0.188 mmol, 2.0 equiv) was added and consumption of **518** was observed. The reaction mixture was cooled to room temperature, concentrated *in vacuo*, and reconstituted in EtOAc. The white precipitate was filtered and the mother liquor (clear and yellow) was concentrated to a non-viscous and orange oil, 116 mg. The crude material was chromatographed on silica gel (hexanes/EtOAc 4:1) to yield **519** (27 mg, 70%) as a colorless oil.

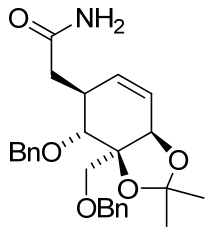
519: R_f = 0.52 (hexanes/EtOAc 3:1); $[\alpha]_D^{18}$ = +119.6 (c = 0.4, $CHCl_3$); IR (film) *ν* 3031, 2987, 2867, 2245, 1496, 1454, 1379, 1251, 1215, 1163, 1092, 1057, 1028, 872, 738, 698, 665 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 7.40 – 7.31 (m, 10H), 6.01 (d, *J* = 9.7 Hz, 1H), 5.85 (d, *J* = 10.0 Hz, 1H), 5.09 (d, *J* = 11.3 Hz, 1H), 4.78 (d, *J* = 3.7 Hz, 1H), 4.65 (d, *J* = 12.1 Hz, 1H), 4.61 (d, *J* = 11.3 Hz, 1H), 4.56 (d, *J* = 12.1 Hz, 1H), 3.81 (d, *J* = 10.3 Hz, 1H), 3.77 (d, *J* = 10.3 Hz, 1H), 3.55 (d, *J* = 9.7 Hz, 1H), 2.69 – 2.66 (m, 2H), 2.31 (dd, *J* = 9.5, 17.6 Hz, 1H), 1.61 (s, 3H), 1.50 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 138.2, 130.8, 128.6, 128.3, 128.0, 125.6, 118.2, 109.5, 84.4, 81.2, 74.6, 74.3, 61.6, 38.3, 28.7, 26.5, 25.9, 20.3, 18.4, -5.4, -5.6; MS (+EI) *m/z* (%) 405 (1), 404 (2), 270 (4), 107 (14), 92 (16), 91 (100), 43 (8); HRMS (+EI) calcd for $C_{25}H_{26}O_4N$: 404.1869. Found: 404.1861; Anal. Calcd for $C_{26}H_{29}O_4N$: C, 74.44; H, 6.97. Found C, 74.21; H, 6.96.



521

2-((*3aS,4R,5S,7aR*)-4-(Benzyloxy)-3a-(benzyloxymethyl)-2,2-dimethyl-3a,4,5,7a-tetrahydrobenzo[*d*][1,3]dioxol-5-yl)-*N*-(3-chloropropyl)acetamide (521).

A white and cloudy mixture of nitrile **519** (190 mg, 0.452 mmol) in aqueous NaOH (4 M, 2.6 mL) and MeOH (2.6 mL) was heated to reflux for 22 h, at which time **519** was consumed as evidenced by analysis by TLC (EtOAc). The reaction mixture was cooled to room temperature and concentrated *in vacuo* to remove MeOH. The aqueous mixture was neutralized with 6 M aqueous HCl and then extracted with Et_2O (4x). The organic phases were combined, dried over $MgSO_4$, and concentrated *in vacuo* to obtain 178 mg (83%) of **520** as a white foam-like solid that was used without further purification.



520

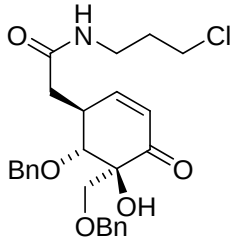
2-((*3aS,4R,5S,7aR*)-4-(Benzyloxy)-3a-(benzyloxymethyl)-2,2-dimethyl-3a,4,5,7a-tetrahydrobenzo[*d*][1,3]dioxol-5-yl)acetamide (520): R_f = 0.11 (hexanes/EtOAc 1:1); $[\alpha]_D^{18}$ = +70.1 (*c* 0.65, $CHCl_3$); FT-IR (film) ν 3604, 3583, 3032, 2986, 2929, 2868, 1731, 1707, 1454, 1379, 1251, 1215, 1163, 1095, 1057, 1029, 873, 736, 697, 665 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.40 – 7.29 (m, 10H), 5.89 (ddd, *J* = 2.4, 4.0, 10 Hz, 1H),

5.78 (d, J = 9.8 Hz, 1H), 5.12 (d, J = 11.2 Hz, 1H), 4.79 (d, J = 3.8 Hz, 1H), 4.68 (d, J = 12.1 Hz, 1H), 4.58 (d, J = 11.9 Hz, 1H), 4.55 (d, J = 11.9 Hz, 1H), 3.93 (d, J = 10.9 Hz, 1H), 3.71 (d, J = 10.7 Hz, 1H), 3.54 (d, J = 9.9 Hz, 1H), 2.76 – 2.69 (m, 2H), 2.30 – 2.21 (m, 1H), 1.60 (s, 3H), 1.51 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.9, 138.4, 138.2, 133.2, 128.3, 128.0, 127.6, 127.5, 127.4, 123.5, 109.3, 84.4, 81.9, 74.6, 73.9, 73.8, 38.0, 36.8, 28.7 26.4; MS (+ESI) *m/z* (%) 422 (25), 421 (100) 333 (7), 315 (14), 255 (25), 237 (15), 181 (20), 165 (14).

To a clear and yellow solution of freshly prepared and crude primary amide **520** (169 mg, 0.386 mmol) in DMF (3.8 mL) at room temperature was added sodium hydride (9 mg, 0.386 mmol, 1.0 equiv) with stirring. To this mixture was added 1-bromo-3-chloropropane (152 μL, 1.54 mmol, 4.0 equiv) and the reaction was monitored by TLC analysis (hexanes/EtOAc 3:1 for **521**; EtOAc for **520**). Upon consumption of **520** the reaction mixture was quenched with aqueous NH_4Cl (satd, 0.2 mL) and further diluted with H_2O (10 mL). The aqueous phase was extracted with Et_2O (3x) and the combined organic phases were washed with H_2O (1x), brine (1x), and dried over $MgSO_4$. The organic phase was concentrated *in vacuo* to yield 169 mg of crude material that was chromatographed on silica gel (hexanes/EtOAc 3:1) and yielded 112 mg (56%) of **521** as a colorless oil.

521: R_f = 0.60 (hexanes/EtOAc 3:1); $[\alpha]_D^{18}$ = +64.7 (*c* 0.75, $CHCl_3$); FT-IR (film) ν 3395, 3030, 2917, 2857, 1734, 1496, 1453, 1379, 1251, 1214, 1154, 1094, 1056, 1028, 872, 736, 697, 665 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.37 – 7.31 (m, 10H), 5.89 (td, J = 3.2, 9.8 Hz, 1H), 5.78 (d, J = 9.9 Hz, 1H), 5.14 (d, J = 11.3 Hz, 1H), 4.81 (d, J = 3.9 Hz, 1H), 4.69 (d, J = 12.1 Hz, 1H), 4.58 (dd, J = 5.1, 11.5 Hz, 2H), 4.19 – 4.10

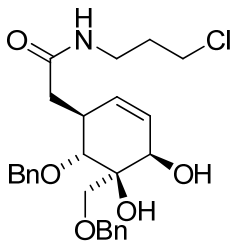
(m, 2H), 3.94 (d, J = 10.7 Hz, 1H), 3.73 (d, J = 10.6 Hz, 1H), 3.58 – 3.50 (m, 2H), 2.78 (dd, J = 8.35, 14.5 Hz, 1H), 2.71 (dd, J = 5.4, 15.4 Hz, 1H), 2.30 (dd, J = 8.3, 15.4 Hz, 1H), 2.01 (quin, J = 5.7 Hz, 2H), 1.62 (s, 3H), 1.52 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 172.1, 138.5, 138.3, 133.4, 128.4, 128.1, 127.7, 127.5, 127.4, 123.5, 109.3, 84.5, 82.0, 74.7, 73.9, 73.8, 67.1, 61.2, 41.2, 38.2, 37.0, 31.5, 28.8, 26.4; MS (+ESI) *m/z* (%) 537 (M+Na, 100), 515 (1), 514 (1), 457 (2), 409 (12), 331 (16), 326 (18), 319 (14), 284 (17), 259 (12), 256 (36), 241 (17), 147 (9); Anal. Calcd for $C_{29}H_{36}O_5NCl$: C, 67.76; H, 7.06. Found C, 67.41; H, 6.83.



388

2-((*1S,5S,6R*)-6-(Benzyloxy)-5-(benzyloxymethyl)-5-hydroxy-4-oxocyclohex-2-enyl)-*N*-(3-chloropropyl)acetamide (388).

To a clear and colorless solution of acetonide **521**(40 mg, 0.075 mmol) in $CHCl_3$ (2 mL) at room temperature was added $FeCl_3 \cdot SiO_2$ (~100 mg) with stirring and the reaction was monitored by TLC analysis (hexanes/EtOAc 1:1). The reaction slurry was filtered and the filtered solid was rinsed with $CHCl_3$ and MeOH. The filtrate was concentrated *in vacuo* to provide 125 mg of crude material. The crude material was passed through a plug of silica gel (hexanes/EtOAc 1:1) to yield 21 mg (62%) of **522** as a colorless oil. The material was used directly for the next step.



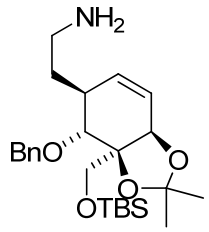
522

2-((*1S,4R,5R,6R*)-6-(Benzyloxy)-5-(benzyloxymethyl)-4,5-dihydroxycyclohex-2-enyl)-*N*-(3-chloropropyl)acetamide (522): R_f = 0.47 (hexanes/EtOAc 1:1); $[\alpha]_D^{18}$ = +11.1 (*c* 0.6, $CHCl_3$); FT-IR (film) ν 3487, 3088, 3030, 2960, 2920, 2872, 1732, 1605, 1586, 1496, 1453, 1391, 1361, 1302, 1258, 1208, 1169, 1094, 1028, 918, 850, 738, 698, 654 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 7.39 – 7.31 (m, 10H), 5.74 (d, *J* = 10.0 Hz, 1H), 5.67 (d, *J* = 10.0 Hz, 1H), 4.73 (d, *J* = 11.7 Hz, 1H), 4.68 (d, *J* = 11.6 Hz, 1H), 4.54 (s, 2H), 4.28 (bs, 1H), 4.23 (t, *J* = 5.9 Hz, 2H), 3.75 (d, *J* = 9.2 Hz, 1H), 3.67 (m, 2H), 3.59 (t, *J* = 6.3 Hz, 2H), 3.10 (bs, 1H), 2.93 (bs, 1H), 2.76 – 2.72 (m, 2H), 2.45 (dd, *J* = 9.7, 19.0 Hz, 1H), 2.08 (quin, *J* = 6.3 Hz, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 172.6, 138.4, 137.5, 130.0, 128.6, 128.3, 128.0, 128.0, 129.0, 127.6, 127.2, 77.8, 74.4, 73.8, 73.2, 71.5, 67.8, 61.2, 41.2, 37.1, 36.7, 31.5; MS (-ESI) *m/z* (%) 519 ($M+HCOO^-$, 100), 113 (20).

To the freshly isolated allylic alcohol **522** (42 mg, 0.088 mmol) in $CHCl_3$ (5 mL) at room temperature was added MnO_2 (153 mg, 1.76 mmol, 20 equiv) as a single portion. The black slurry was stirred at room temperature and monitored by TLC analysis (hexanes/EtOAc 1:1). Another portion of MnO_2 (76 mg, 0.88 mmol, 10 equiv) was added to the reaction mixture after 15 h. The reaction was stopped with **522** still present in the mixture and the reaction was filtered through a pad of Celite. The filtrate was concentrated *in vacuo* to yield 40 mg of a colorless oil. The crude material was subjected

to chromatography on silica gel (hexanes/EtOAc 3:1 → 1:1) to yield 23 mg (56%) of enone **388** and 12 mg (28%) recovered **522**.

388: R_f = 0.72 (hexanes/EtOAc 1:1); $[\alpha]_D^{18}$ = +146.9 (*c* 0.25, $CHCl_3$); FT-IR (film) ν 3467, 2956, 2919, 2857, 1732, 1687, 1495, 1453, 1363, 1253, 1166, 1096, 1027, 738, 698, 665 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 7.38 – 7.26 (m, 10H), 6.81 (d, *J* = 10.3 Hz, 1H), 6.17 (dd, *J* = 3.4, 11.0 Hz, 1H), 5.11 (d, *J* = 11.3 Hz, 1H), 4.61 (d, *J* = 11.2 Hz, 1H), 4.57 (d, *J* = 12.4 Hz, 1H), 4.50 (d, *J* = 12.3 Hz, 1H), 4.18 (t, *J* = 5.9 Hz, 2H), 4.15 (s, 1H), 3.93 (d, *J* = 9.8 Hz, 1H), 3.83 (d, *J* = 9.7 Hz, 1H), 3.71 (d, *J* = 9.8 Hz, 1H), 3.54 (dt, *J* = 1.5, 9.3 Hz, 2H), 3.15 – 3.10 (m, 1H), 2.72 (dd, *J* = 4.8, 16.4 Hz, 1H), 2.40 (dd, *J* = 8.0, 16.3 Hz, 1H), 2.04 (quin, *J* = 6.2 Hz, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 199.8, 171.3, 150.8, 138.0, 137.7, 128.4, 128.4, 127.9, 127.5, 127.4, 126.6, 83.1, 81.2, 74.9, 73.7, 70.9, 61.5, 41.1, 39.2, 35.8, 31.3; MS (+EI) *m/z* (%) 471 (1), 435 (3), 350 (2), 344 (9), 222 (5), 208 (6), 207 (5), 152 (6), 123 (10), 111 (12), 107 (19), 100 (10), 99 (37), 97 (20), 95 (13), 92 (23), 91 (100) 72 (20), 71 (23), 59 (53), 57 (40), 55 (39), 43 (57), 41 (31); HRMS (+EI) calcd for $C_{26}H_{30}O_5NCl$: 471.1803. Found: 471.1812.

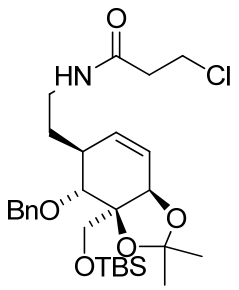


512

2-((*3aS,4R,5S,7aR*)-4-(Benzyloxy)-3a-((*tert*-butyldimethylsilyloxy)methyl)-2,2-dimethyl-3a,4,5,7a-tetrahydrobenzo[*d*][1,3]dioxol-5-yl)ethanamine (512).

To a slurry of $LiAlH_4$ (10 mg, 0.270 mmol, 2.0 equiv) in Et_2O (1.4 mL) at room temperature was added a solution of nitrile **510** (60 mg, 0.135 mmol) in Et_2O (0.7 mL) in three portions. The reaction was monitored by TLC analysis (hexanes/EtOAc 2:1) and upon consumption of **510** the reaction mixture was quenched with Rochelle's salt (satd, 0.2 mL), the mother liquor was decanted from the inorganic precipitates and dried over Na_2SO_4 to yield 56 mg (93%) of **512** as a white solid.

512: R_f = 0.46 (EtOAc/MeOH 4:1); mp = 97 – 101 ˚C (Et_2O); $[\alpha]_D^{18}$ = +64.2 (*c* 0.4, $CHCl_3$); FT-IR (film) ν 3362, 2926, 2855, 1570, 1461, 1378, 1360, 1251, 1213, 1092, 1019, 852, 778, 741, 665 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 7.40 (d, *J* = 7.1 Hz, 1H), 7.36 (t, *J* = 7.5 Hz, 2H), 7.30 (t, *J* = 7.5 Hz, 1H), 5.85 (ddd, *J* = 2.6, 4.1, 9.8 Hz, 1H), 5.80 (d, *J* = 10.2 Hz, 1H), 5.11 (d, *J* = 11.3 Hz, 1H), 4.69 (d, *J* = 3.8 Hz, 1H), 4.60 (d, *J* = 11.3 Hz, 1H), 4.04 (d, *J* = 11.0 Hz, 1H), 3.73 (d, *J* = 11.3 Hz, 1H), 3.51 (d, *J* = 10.0 Hz, 1H), 2.78 – 2.73 (m, 1H), 2.70 – 2.66 (m, 1H), 2.27 (t, *J* = 8.7 Hz, 1H), 1.86 – 1.80 (m, 1H), 1.59 (s, 3H), 1.48 (s, 3H), 0.91 (s, 9H), 0.08 (s, 3H), 0.07 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 138.8, 134.4, 128.4, 128.2, 127.7, 122.5, 108.8, 85.3, 82.5, 74.9, 73.1, 60.5, 39.4, 39.0, 35.8, 28.8, 26.7, 26.0, 18.4, -5.3, -5.6; MS (+EI) *m/z* (%) 448 (9), 390 (7), 115 (7), 91 (100), 89 (18), 75 (25), 73 (93), 59 (16), 43 (12), 30 (25); HRMS (+EI) calcd for $C_{25}H_{42}O_4NSi$: 448.2814. Found: 448.2883; Anal calcd for $C_{25}H_{41}NO_4Si$: C, 67.07; H, 9.23. Found: C, 66.17; H, 9.16.



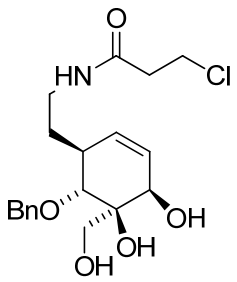
513

***N*-(2-((*3aS,4R,5S,7aR*)-4-(Benzyloxy)-3a-((*tert*-butyldimethylsilyloxy)methyl)-2,2-dimethyl-3a,4,5,7a-tetrahydrobenzo[*d*][1,3]dioxol-5-yl)ethyl)-3-chloropropanamide (513)**.

To a clear and colorless solution of amine **512** (48 mg, 0.108 mmol) in methylene chloride (1 mL) at 5 ˚C was added Et_3N (30 μL, 0.216 mmol, 2.0 equiv) followed by 3-chloropropionyl chloride (20 μL, 0.216 mmol, 2.0 equiv). The reaction was monitored by TLC analysis (hexanes/EtOAc 2:1) and the mixture quenched with aqueous NH_4Cl (satd, 0.25 mL). The phases were separated and the organic phase was collected, washed with H_2O (1x), brine (1x), and dried over Na_2SO_4. The organic phase was concentrated to a yellow oil, 50 mg, that was chromatographed on silica gel (hexanes/EtOAc 2:1) and yielded 37 mg (64%) of **513** as a colorless oil.

513: R_f = 0.19 (hexanes/EtOAc 2:1); $[\alpha]_D^{18}$ = +12.5 (*c* 0.3, $CHCl_3$); FT-IR (film) ν 3402, 2919, 2861, 1731, 1458, 1371, 1365, 1214, 1090, 1051, 1031, 873, 735, 662 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 7.43 (d, *J* = 7.7 Hz, 2H), 7.36 (t, *J* = 7.4 Hz, 1H), 7.29 (t, *J* = 7.2 Hz, 1H), 6.19 – 6.12 (m, 1H), 5.88 (d, *J* = 10.0 Hz, 1H), 5.86 – 5.82 (m, 1H), 5.53 (dd, *J* = 3.4, 9.0 Hz, 1H), 5.10 (d, *J* = 11.2 Hz, 1H), 4.67 – 4.66 (rotamer; m, 2H), 4.01 (dd, *J* = 11.1, 7.6 Hz, 1H), 3.82 (dd, *J* = 11.0, 13.6 Hz, 2H), 3.54 (dd, *J* = 10.0, 5.4 Hz,

1H), 3.39 – 3.29 (rotamer; m, 1H), 2.34 (bs, 1H), 2.03 – 1.96 (rotamer; m, 1H), 1.53 (s, 3H), 1.43 (s, 3H), 0.91 (s, 9H), 0.10 (s, 3H), 0.09 (s, 3H); ^{13}C NMR (150 MHz, acetone-d_6) 168.6, 164.5, 139.3, 133.7, 133.6, 131.9, 128.2, 127.9, 127.3, 124.2, 123.1, 122.9, 108.5, 85.0, 84.9, 82.6, 82.5, 74.5, 73.5, 73.4, 61.0, 60.8, 40.6, 38.9, 38.8, 38.8, 36.4, 36.8, 31.7, 28.3, 26.2, 25.5, 18.1, -6.0, -6.1; MS (+EI) *m/z* (%) 408 (1), 278 (5), 262 (7), 207 (12), 105 (8), 91 (100), 77 (9), 75 (23), 73 (17), 55 (22), 43 (14), 41 (8); HRMS (+EI) calcd for $C_{27}H_{41}O_5NClSi$: 522.2423. Found: 522.2442.

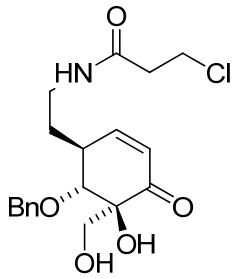


514

***N*-(2-((*1S,4R,5R,6R*)-6-(Benzyloxy)-4,5-dihydroxy-5-(hydroxymethyl)cyclohex-2-enyl)ethyl)-3-chloropropanamide (514)**.

To a crude and hazy solution of acetonide **514** (1.59 g, 2.97 mmol; based on theoretical from a previous step) in MeOH (30 mL) at room temperature was added conc. HCl (1.5 mL) with stirring. The reaction was monitored by TLC (EtOAc) and **514** was consumed after ca. 3 h. The mixture was neutralized with aqueous NaOH and filtered. The filtrate was concentrated to an oily white solid that was triturated with EtOAc, filtered again, and the filtrate concentrated *in vacuo* to yield a yellow-white foam, 1.17 g. The crude material was chromatographed on deactivated silica gel (hexanes/EtOAc 1:1 → EtOAc → MeOH flush) to yield 0.70 g (61%) of **514** as a colorless oil.

514: R_f = 0.22 (EtOAc); $[\alpha]_D^{18}$ = +41.1 (*c* 1.0, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.41 – 7.25 (m, 5H), 5.75 (s, 2H), 4.98 (d, *J* = 11.1 Hz, 1H), 4.64 (d, *J* = 11.1 Hz, 1H), 4.15 – 4.08 (m, 1H), 3.90 (d, *J* = 11.5 Hz, 1H), 3.78 (t, *J* = 6.4 Hz, 1H), 3.68 (d, *J* = 7.4 Hz, 1H), 3.53 (d, *J* = 11.4 Hz, 1H), 3.28 (d, *J* = 7.6 Hz, 1H), 2.61 (t, *J* = 6.3 Hz, 2H), 2.22 (dd, *J* = 7.5, 12.8 Hz, 1H), 1.95 – 1.84 (m, 1H), 1.68 – 1.57 (m, 1H); ^{13}C NMR (75 MHz, MeOH-d_4) δ 170.9, 138.9, 131.1, 127.9, 127.8, 127.2, 126.4, 79.8, 75.6, 74.4, 67.1, 61.9, 39.8, 38.9, 38.6, 36.6, 31.7; MS (+FAB) *m/z* (%) 384 (7), 214 (23), 176 (9), 155 (14), 138 (19), 137 (43), 136 (41), 121 (13), 120 (15), 108 (10), 107 (28), 105 (14), 95 (10), 91 (100), 81 (10), 77 (30), 69 (14), 57 (18), 55 (23), 43 (20), 41 (17), 39 (14); HRMS (+FAB) calcd for $C_{19}H_{27}O_5NCl$: 384.1565. Found: 384.15778.



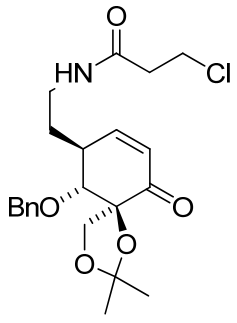
515

***N*-(2-((*1S,5S,6R*)-6-(Benzyloxy)-5-hydroxy-5-(hydroxymethyl)-4-oxocyclohex-2-enyl)ethyl)-3-chloropropanamide (515)**.

To a clear and colorless solution of triol **514** (0.68 g, 1.77 mmol) in $CHCl_3$ (25 mL) at room temperature was added MnO_2 (2.30 g, 26.5 mmol, 15 equiv) over two minutes. The reaction mixture was stirred at room temperature and monitored by TLC (EtOAc). After 22 h another portion of MnO_2 (1.5 g, 17.7 mmol, 10 equiv) was added to the reaction mixture. After another 5 hours the reaction mixture was filtered (**514** still remained)

through a pad of acid washed Celite. The pad of Celite was heavily rinsed with $CHCl_3$ (~300 mL). The brown colored filtrate was concentrated *in vacuo* to provide 477 mg of crude material that was purified by chromatography on silica gel (EtOAc) to yield 352 mg (52%) of enone **515** as an orange oil.

515: R_f = 0.13 (hexanes/EtOAc 1:1.5); $[\alpha]_D^{18}$ = +118.3 (*c* 0.25, $CHCl_3$); FT-IR (film) ν 3583, 3306, 2926, 2868, 1679, 1648, 1552, 1453, 1211, 1099, 1072, 745, 665 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 7.40 (d, *J* = 4.3 Hz, 2H), 7.39 – 7.34 (m, 4H), 6.89 (d, *J* = 10.2 Hz, 1H), 6.16 (dd, *J* = 2.7, 10.5 Hz, 1H), 5.71 (bs, 1H), 5.16 (d, *J* = 11.0 Hz, 1H), 4.64 (d, *J* = 11.1 Hz, 1H), 4.26 (bs, 1H), 4.14 (d, *J* = 11.6 Hz, 1H), 3.81 (d, *J* = 11.6 Hz, 1H), 3.80 – 3.71 (m, 2H), 3.69 (d, *J* = 9.4 Hz, 1H), 3.50 – 3.45 (m, *J* = 7.1 Hz, 1H), 3.09 – 3.04 (m, *J* = 6.4 Hz, 1H), 2.66 (d, *J* = 3.7 Hz, 1H), 2.51 – 2.44 (m, 2H), 2.31 (bs, 1H), 1.87 – 1.82 (m, *J* = 6.8 Hz, 1H), 1.75 – 1.69 (m, *J* = 6.9 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 200.0, 169.6, 151.9, 137.7, 128.7, 128.6, 128.2, 125.8, 83.9, 81.8, 75.0, 63.9, 40.2, 39.8, 39.5, 36.7, 31.5;

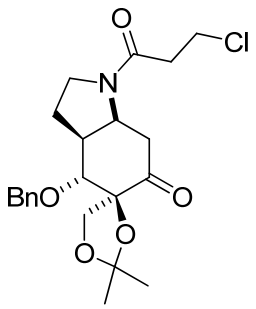


387

***N*-(2-((*5S,6R,7S*)-6-(Benzyloxy)-2,2-dimethyl-10-oxo-1,3-dioxaspiro[4.5]dec-8-en-7-yl)ethyl)-3-chloropropanamide (387)**.

To a clear and orange solution of enone **515** (312 mg, 0.817 mmol) in acetone (8 mL) at room temperature was added 2,2-DMP (3.7 mL) and a crystal of *p*-TsOH with stirring. The reaction was monitored by TLC (EtOAc) and **515** was consumed after 1.5 h and the reaction mixture was quenched with aqueous $NaHCO_3$ (satd, 5 drops). The acetone and 2,2-DMP were removed *in vacuo* and the crude material was reconstituted in methylene chloride, washed with H_2O (1x), brine (1x), and dried over Na_2SO_4. The organic phase was then concentrated to 321 mg of crude orange oil that was purified by chromatography to yield 286 mg (83%) of enone **387** as a light brown oil.

387: R_f = 0.13 (hexanes/EtOAc 1:1.5); $[\alpha]_D^{18}$ = +130.4 (*c* 0.65, $CHCl_3$); FT-IR (film) ν 3309, 2984, 2936, 2875, 1694, 1651, 1547, 1454, 1380, 1370, 1256, 1222, 1101, 1069, 860, 750, 665 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 7.42 – 7.36 (m, 5H), 6.79 (dd, *J* = 2.6, 10.2 Hz, 1H), 6.07 (dd, *J* = 3.6, 11.2 Hz, 1H), 5.43 (t, *J* = 6.6 Hz, 1H), 5.04 (d, *J* = 11.6 Hz, 1H), 4.65 (d, *J* = 11.7 Hz, 1H), 4.40 (d, *J* = 8.7 Hz, 1H), 4.05 (d, *J* = 8.7 Hz, 1H), 3.83 – 3.75 (m, 3H), 3.34 (ddd, *J* = 7.2 Hz, 1H), 3.03 – 2.97 (m, *J* = 6.4 Hz, 1H), 2.56 – 2.49 (m, *J* = 6.2 Hz, 3H), 1.87 – 1.81 (m, *J* = 6.9 Hz, 1H), 1.68 (s, 3H), 1.64 – 1.58 (m, *J* = 6.1 Hz, 2H), 1.52 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 197.6, 169.4, 149.5, 137.8, 128.6, 128.5, 128.1, 127.4, 112.2, 88.3, 79.4, 75.5, 66.4, 40.3, 40.2, 39.6, 36.6, 30.9, 26.4, 26.0; MS (+EI) *m/z* (%) 406 (1), 385 (1), 278 (6), 277 (5), 255 (8), 236 (9), 220 (14), 219 (18), 150 (8), 129 (43), 122 (6), 108 (10), 98 (7), 92 (15), 91 (100), 55 (34); HRMS (+EI) calcd for $C_{22}H_{28}O_5NCl$: 421.1652. Found: 421.16560.



523

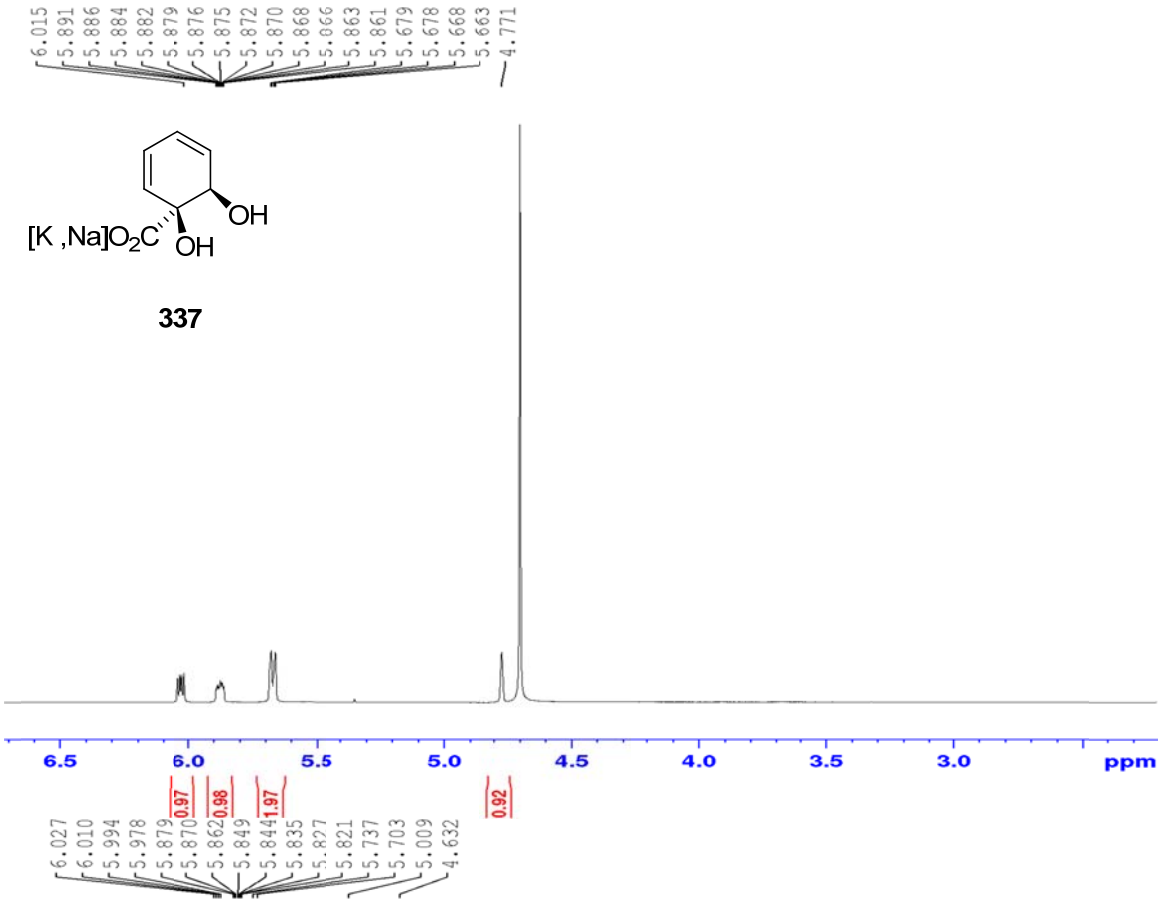
(*3a'S,4S,4'R,7a'S*)-4'-(Benzyloxy)-1'-(3-chloropropanoyl)-2,2-(3a'*S*,4*S*,4'*R*,7a'*S*)-4'-(benzyloxy)-1'-(3-chloropropanoyl)-2,2-dimethylhexahydrospiro[[1,3]dioxolane-4,5'-indol]-6'(1'H)-one (523).

To a clear and colorless solution of enone **387** (22 mg, 0.052 mmol) in THF (5.2 mL) at 5 ˚C was added pentane-washed NaH (1 mg, 0.052, 1.0 equiv) with stirring. The reaction was monitored carefully by TLC (hexanes/EtOAc 1:1.5) and slowly warmed to room temperature. After 1 h the reaction was quenched with aqueous NH_4Cl (sat'd, 10 drops). The reaction mixture was diluted with EtOAc and stirred with Na_2SO_4 for several minutes. The reaction mixture was filtered, the filtrate was concentrated *in vacuo* and dried under vacuum to yield 22 mg of crude material. The crude material was chromatographed on silica gel (hexanes/EtOAc 1:1 → 1:1.5) and provided 13 mg (62%) of **523** and 4 mg (18%) of **524**.

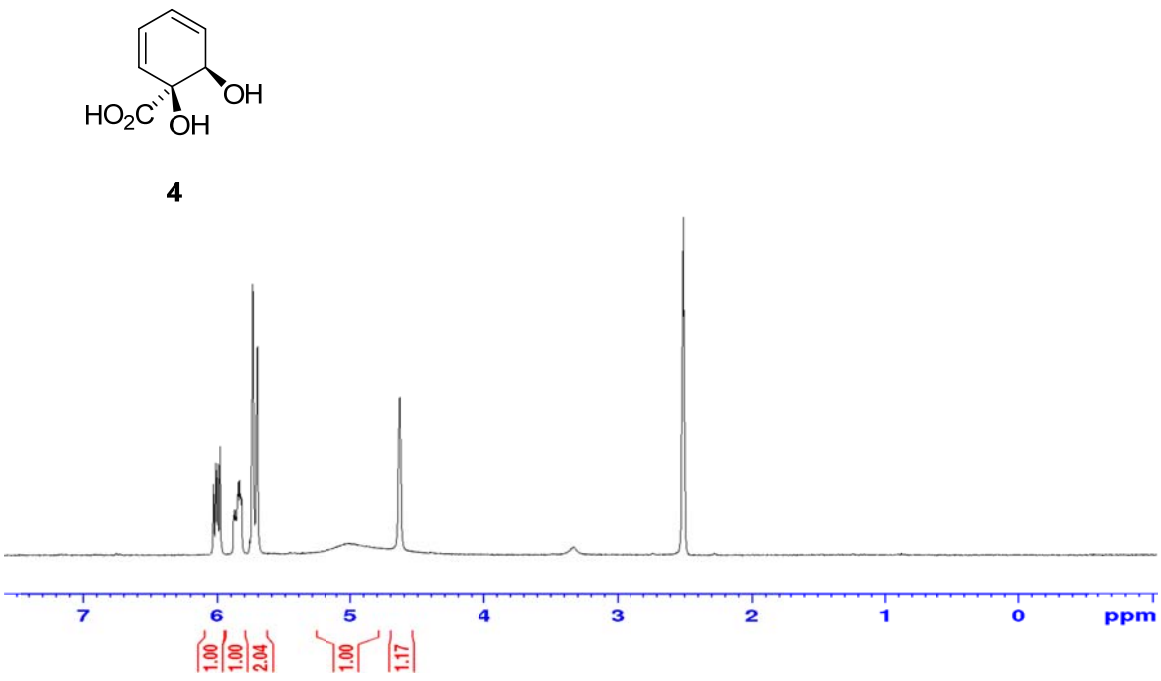
523: R_f = 0.51 (hexanes/EtOAc 1:1.5); $[\alpha]_D^{18}$ = +35.2 (*c* 0.3, $CHCl_3$); (Unresolved rotameric signals) ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.38 – 7.36 (m, 2H), 7.30 – 7.32 (m, 3H), 4.65 (d, *J* = 11.3 Hz, 1H), 4.58 (d, *J* = 11.4 Hz, 1H), 4.34 (d, *J* = 9.2 Hz, 1H), 4.25 (dd, *J* = 7.5, 15.7 Hz, 1H), 3.96 (dd, *J* = 4.2, 16.7 Hz, 1H), 3.83 (dd, *J* = 9.1, 19.9 Hz, 1H), 3.78 (t, *J* = 7.1 Hz, 1H), 3.65 (t, *J* = 9.2 Hz, 1H), 3.50 – 3.42 (m, 1H), 2.80 – 2.62

(m, 4H), 2.42 – 2.35 (m, 1H), 2.03 – 2.00 (m, 1H), 1.33 (s, 3H), 1.28 (s, 3H); (Unresolved rotameric signals) ^{13}C NMR (150 MHz, DMSO-d_6) δ 204.7, 168.4, 167.9, 137.1, 136.9, 128.6, 128.6, 128.3, 128.1, 127.8, 127.7, 111.7, 111.5, 84.3, 83.7, 80.8, 80.3, 72.4, 72.1, 67.2, 67.2, 56.8, 55.4, 46.1, 45.0, 41.7, 40.8, 40.2, 40.0, 39.7, 39.4, 37.4, 37.1, 26.5, 26.4, 26.3, 26.3, 26.1, 23.8; MS (+EI) *m/z* (%) 421 (1), 278 (4), 272 (5), 221 (4), 182 (4), 129 (6), 98 (16), 92 (10), 91 (100) 55 (23), 43 (14), 41 (6); HRMS (+EI) calcd for $C_{22}H_{28}O_5NCl$: 421.1658. Found: 421.1656.

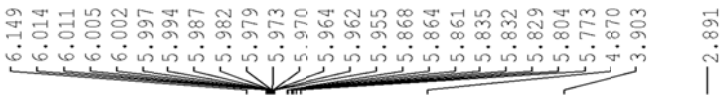
6.0 Selected Spectra



337



4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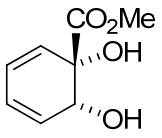
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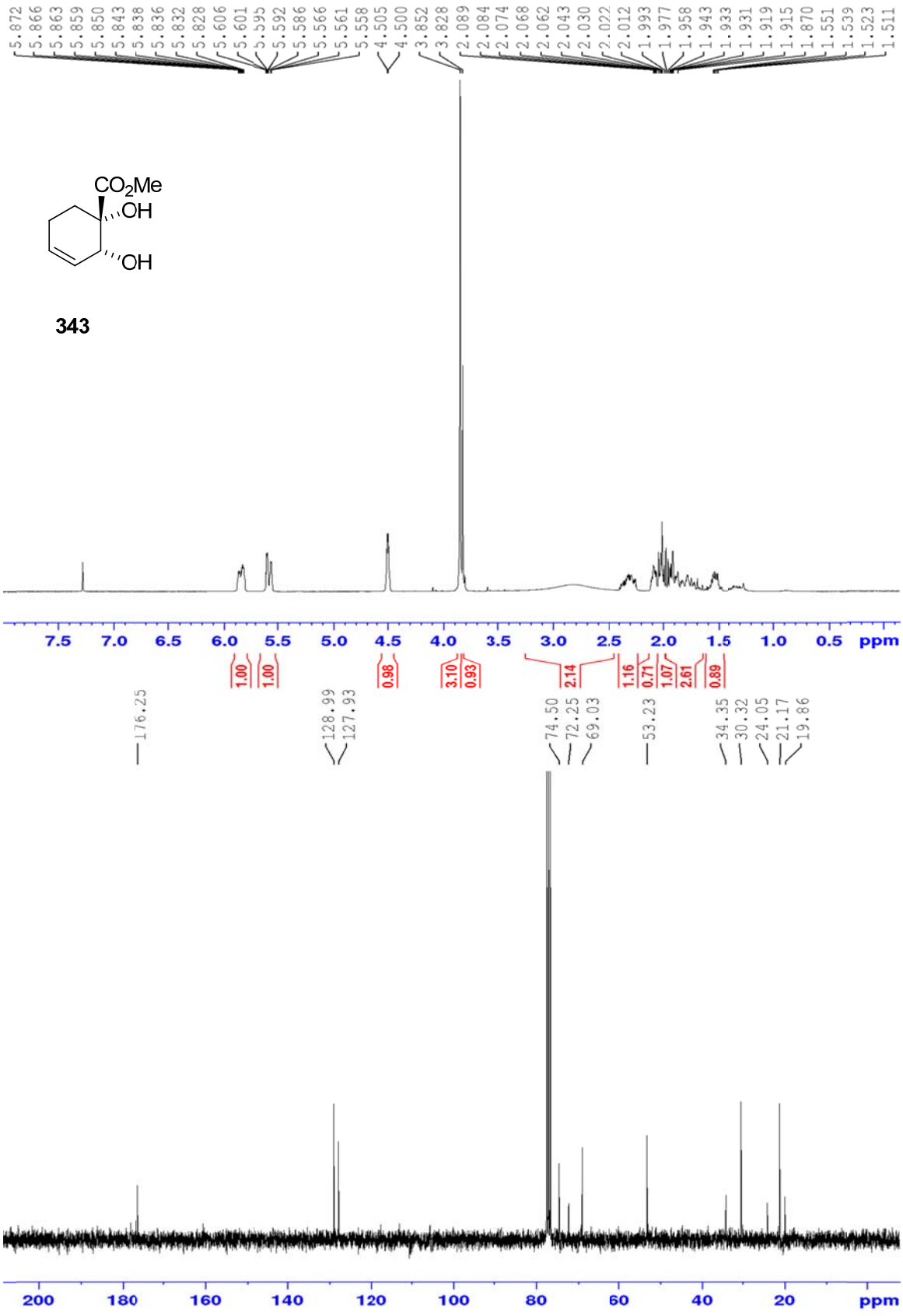
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1.00
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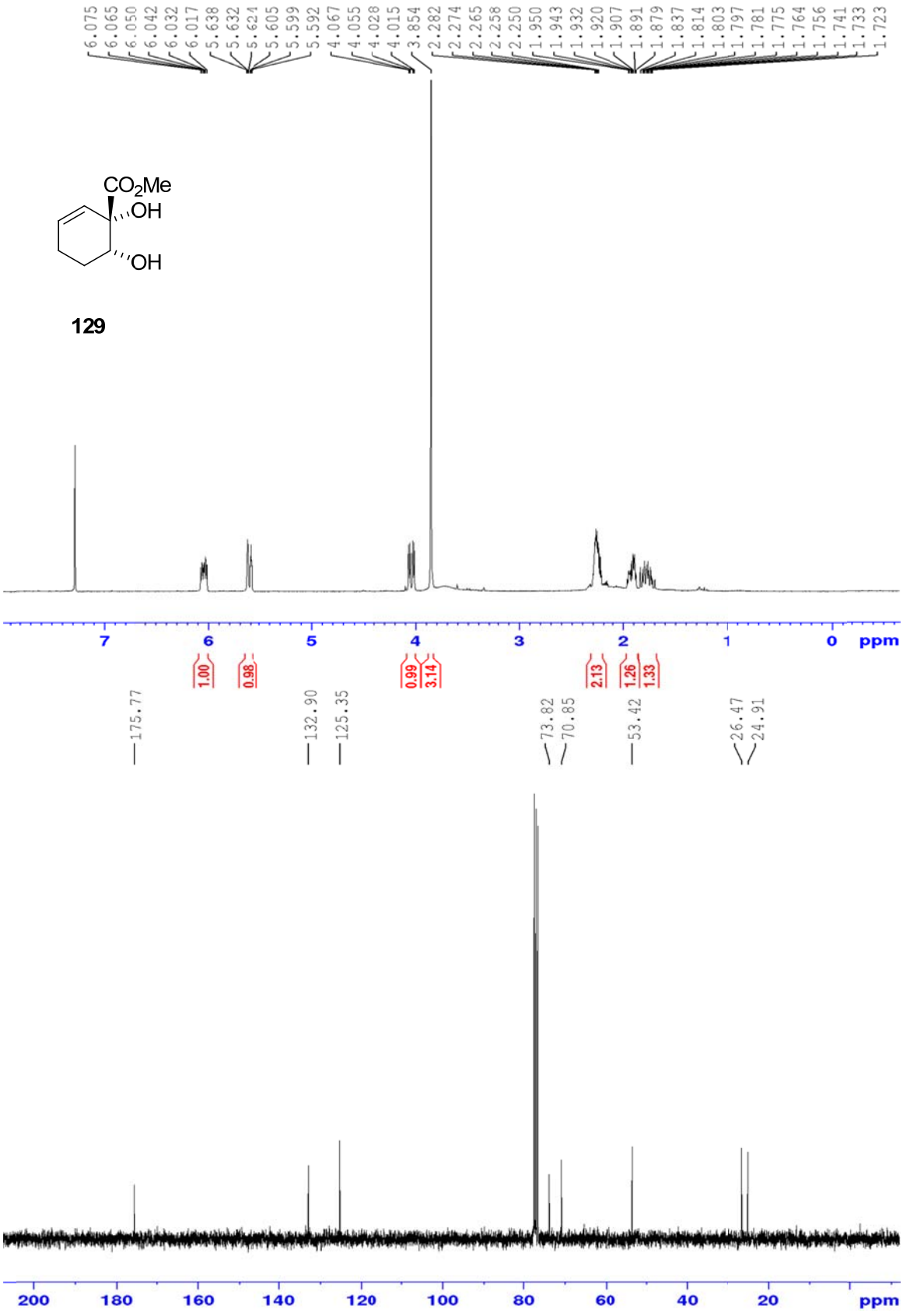
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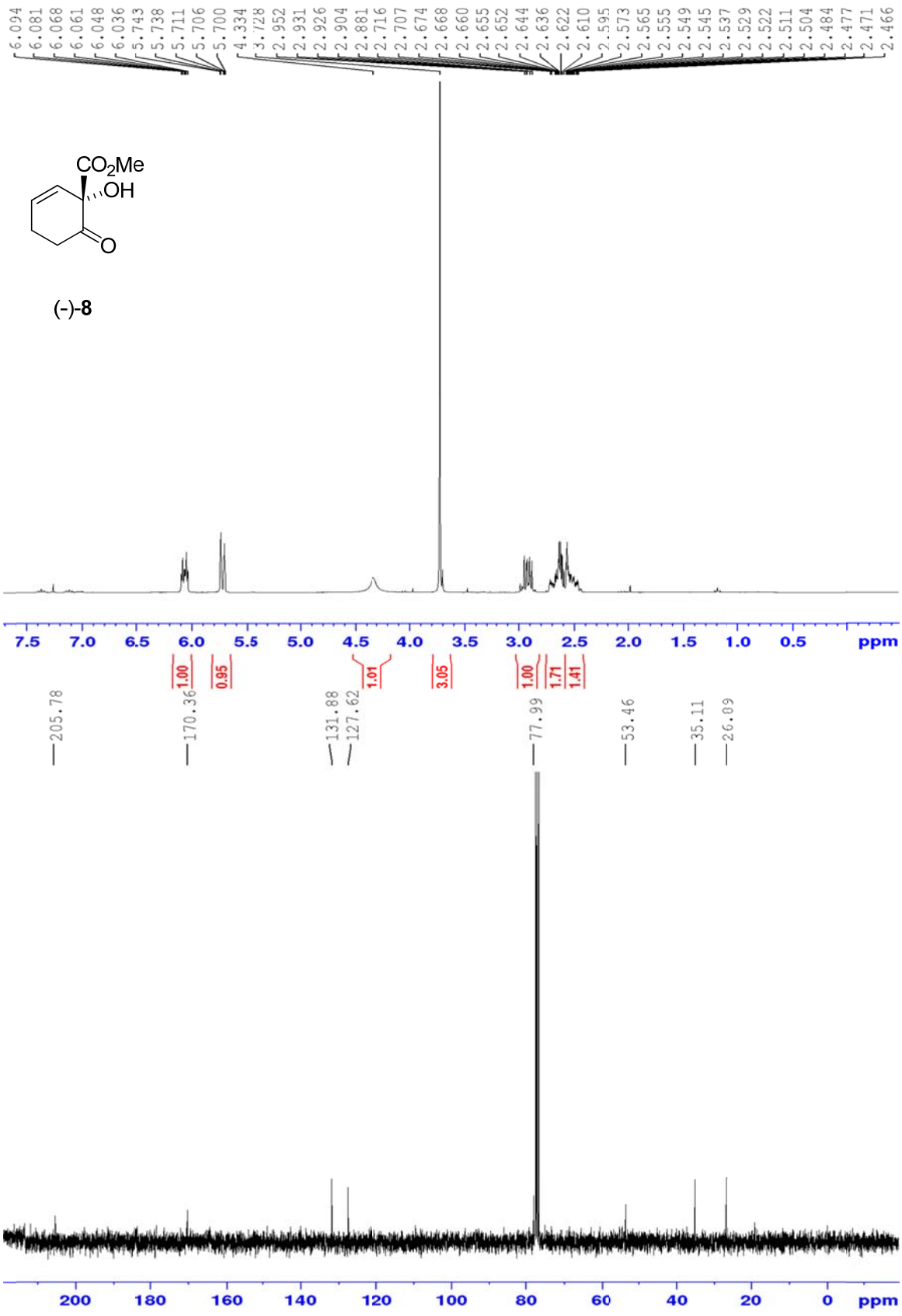
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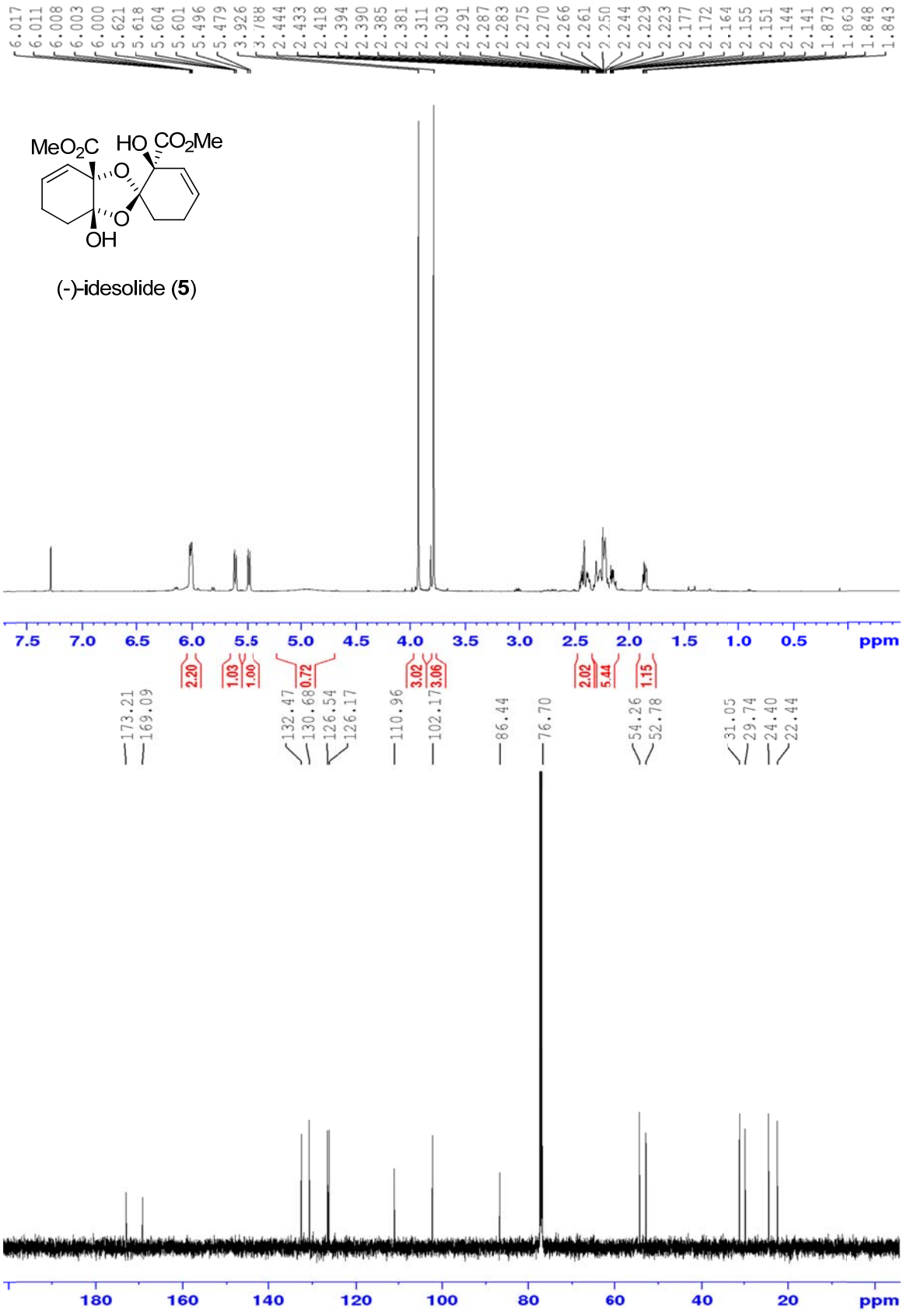
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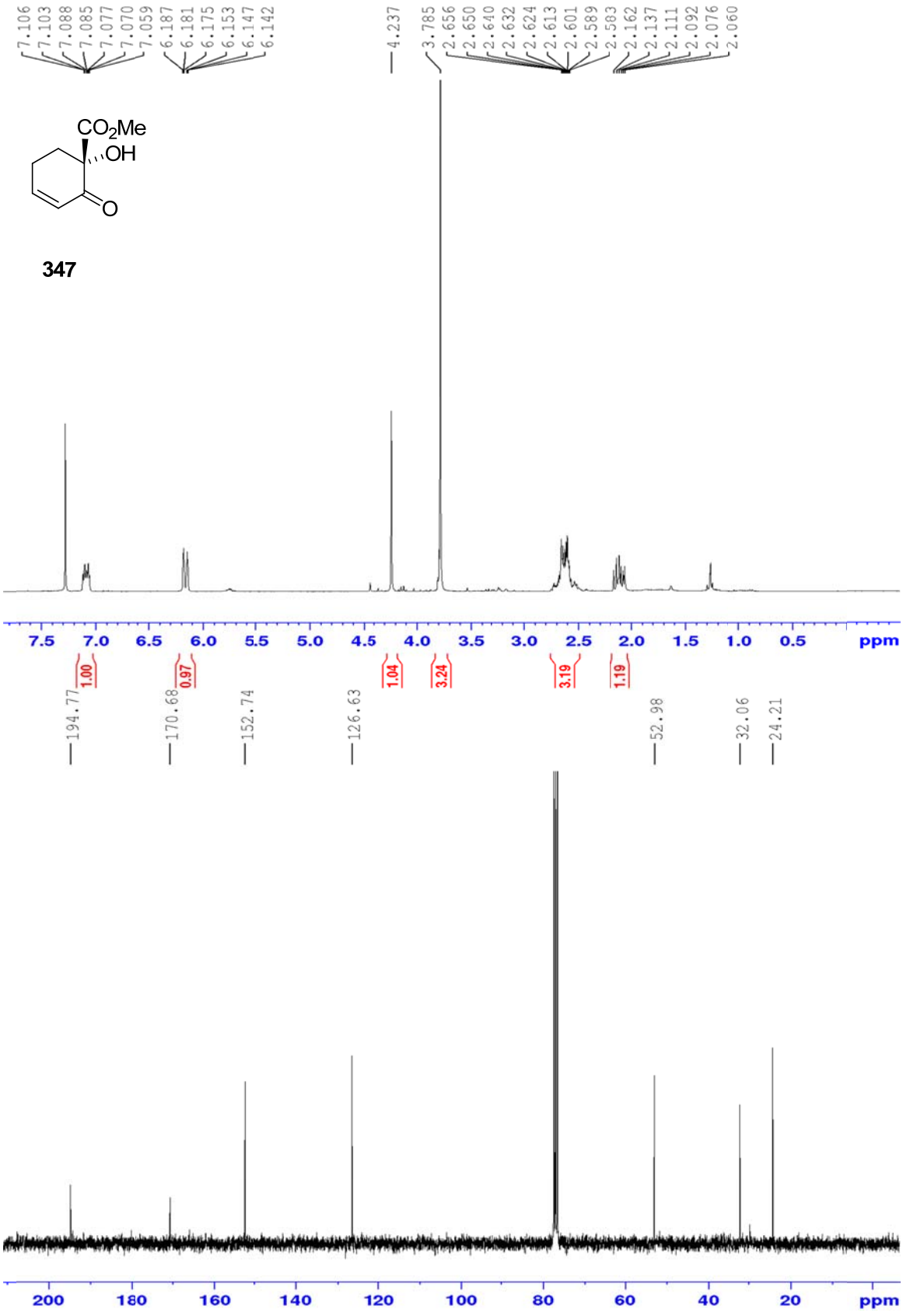
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24.21
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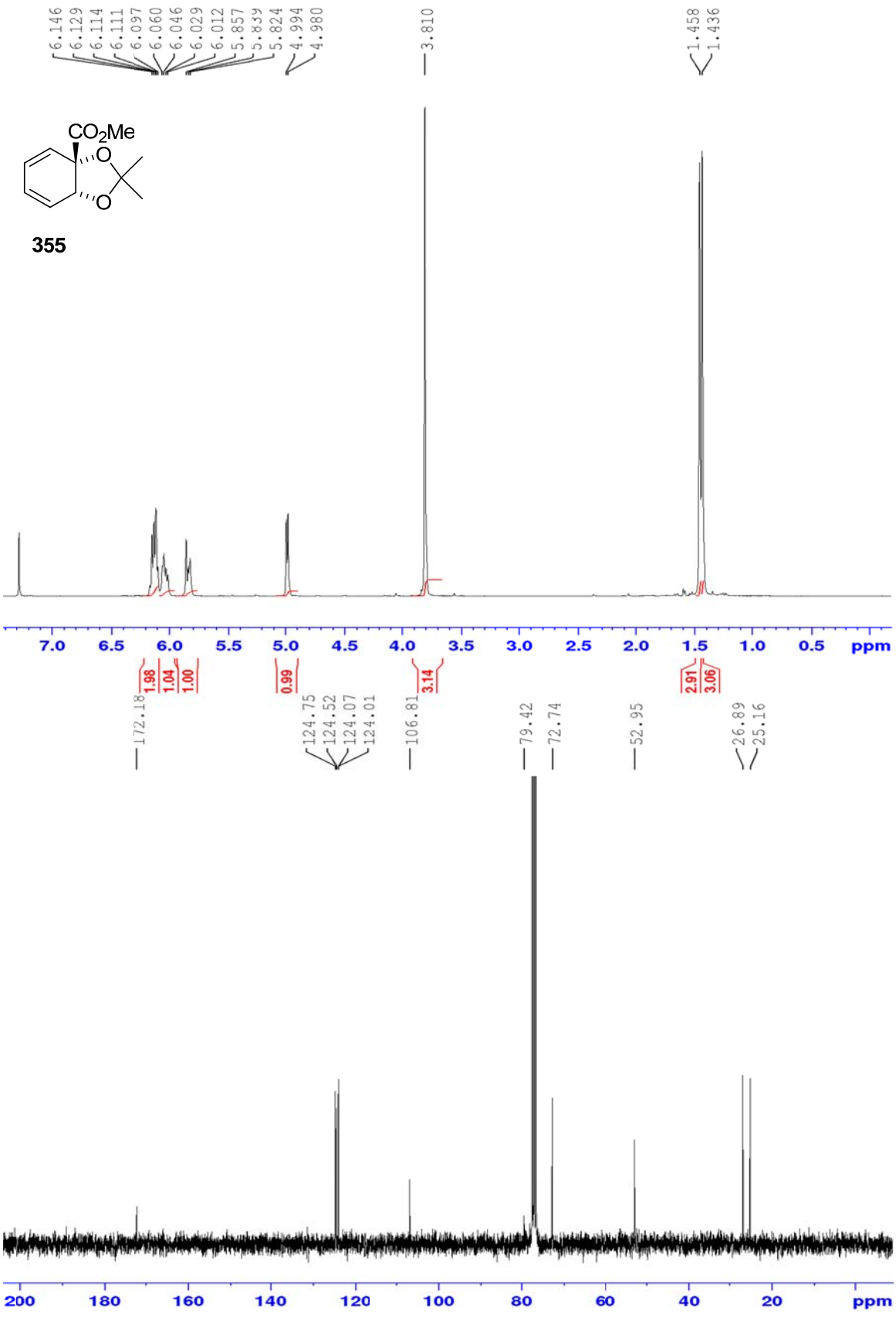
160
140
120
100
80
60
40
20
ppm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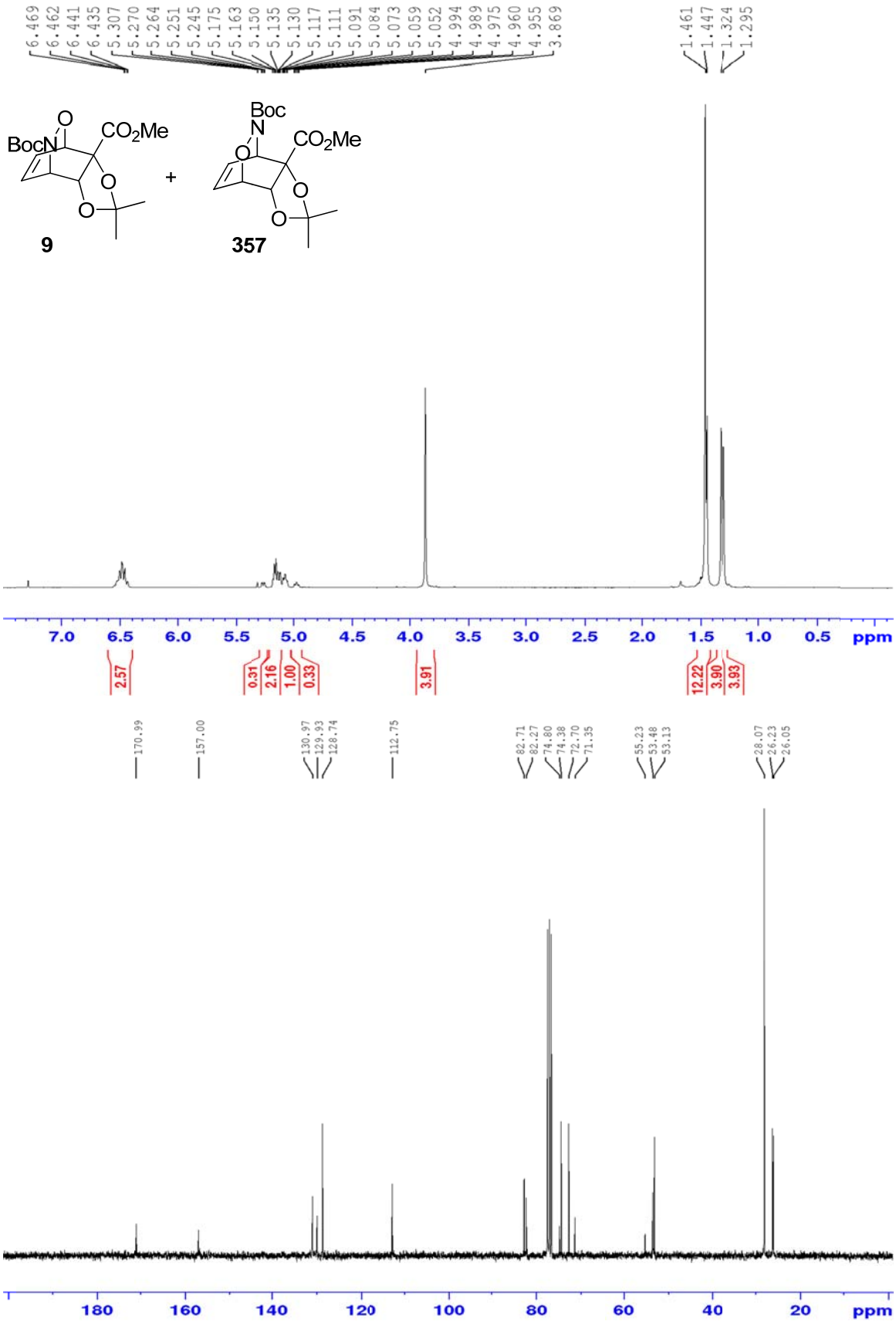
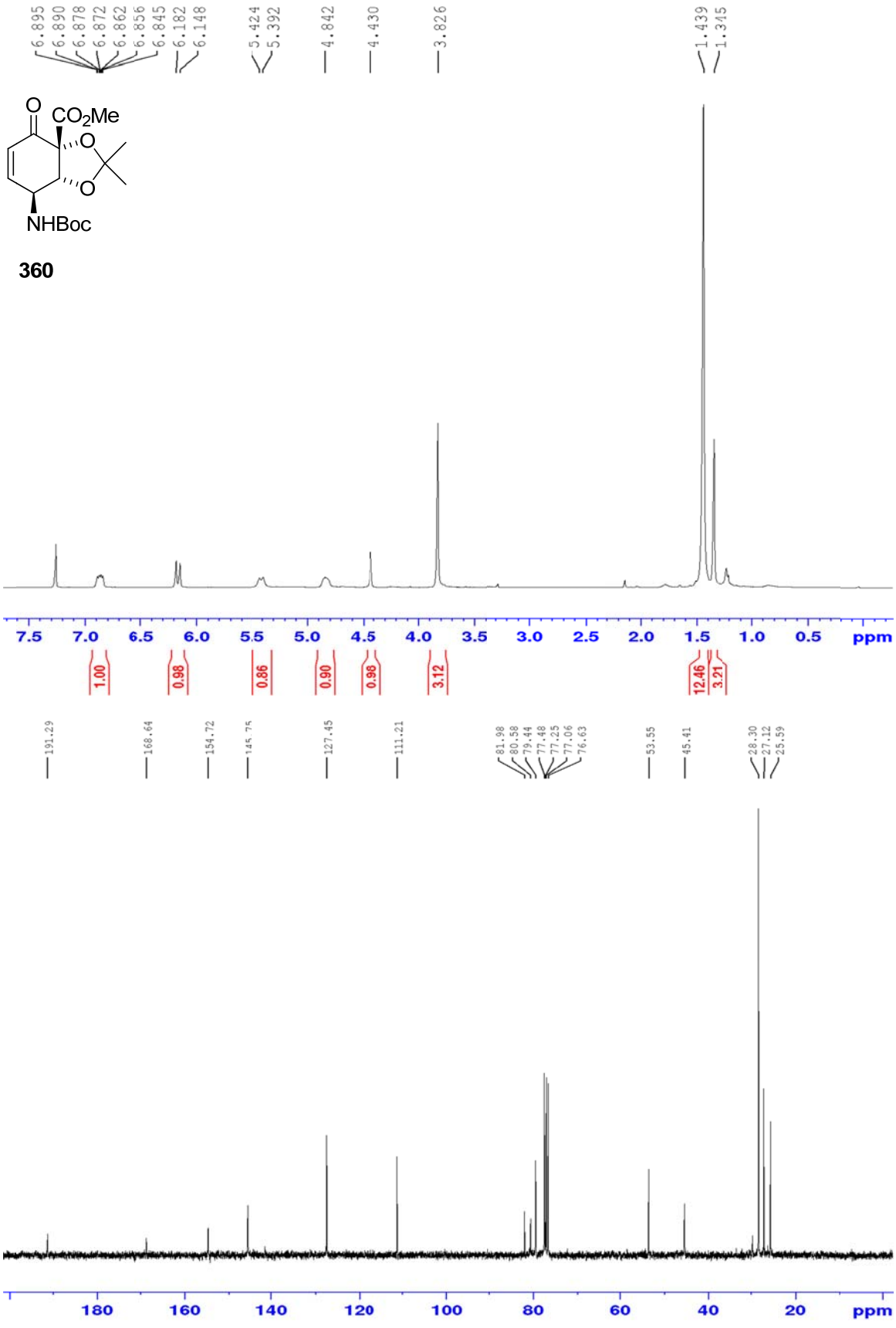
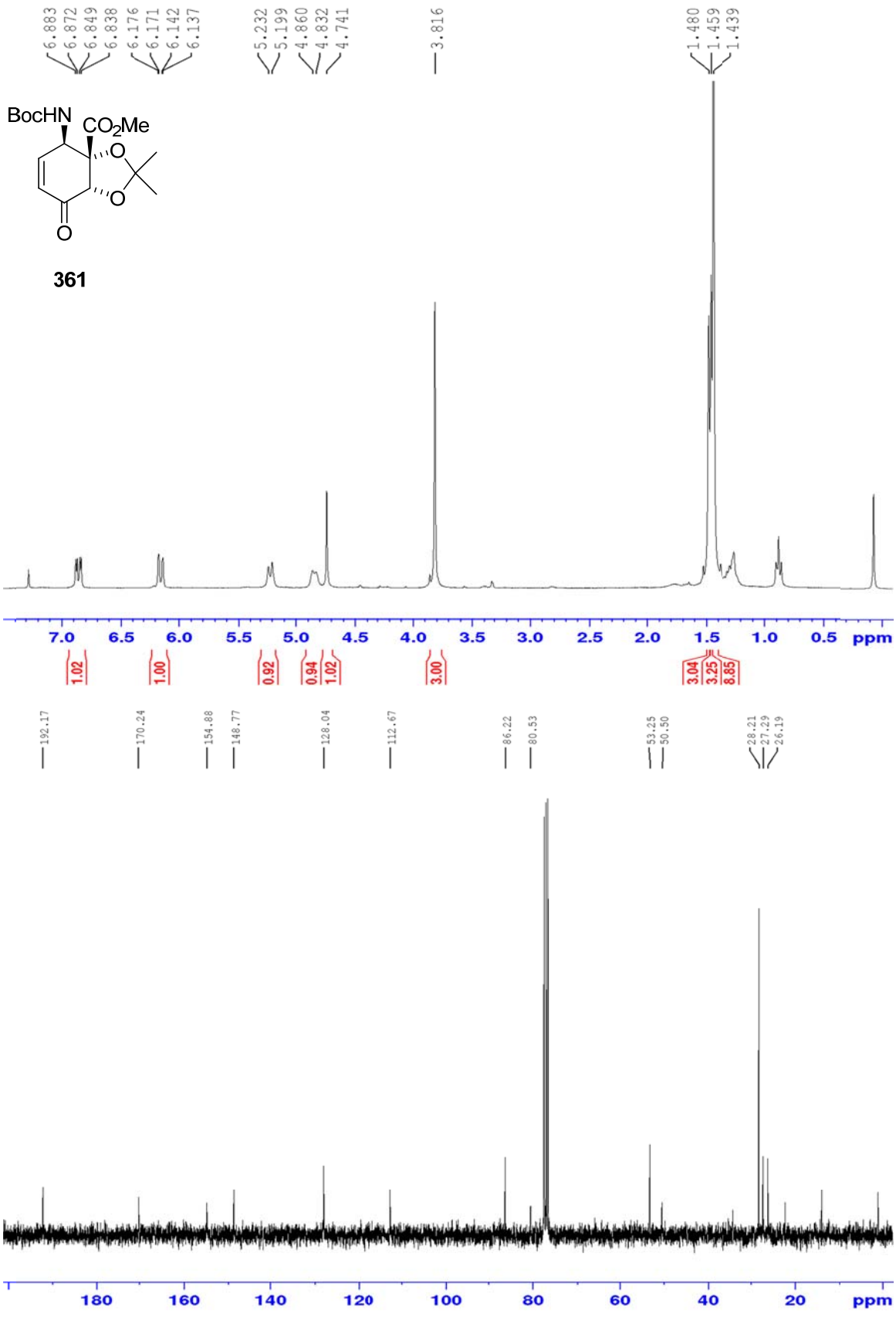
6.129
6.114
6.111
6.097
6.060
6.046
6.029
6.012
5.857
5.839
5.824
4.994
4.980
3.810
1.458
1.436
CO2Me
O
O
355
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
1.98
1.04
1.00
0.99
3.14
2.91
3.06
172.18
124.75
124.52
124.07
124.01
106.81
79.42
72.74
52.95
26.89
25.16
200
180
160
140
120
100
80
60
40
20
ppm

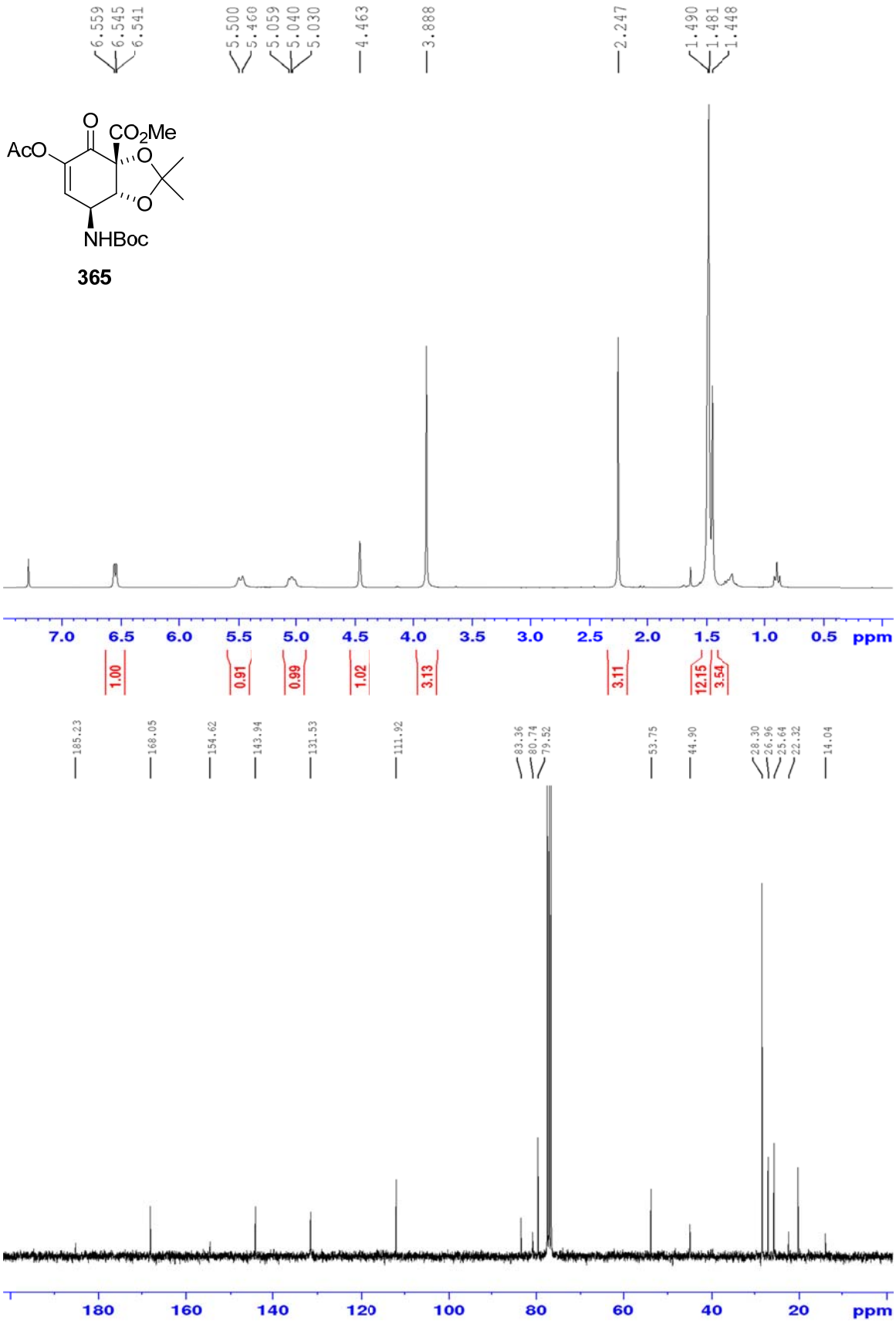

6.469
6.462
6.441
6.435
5.307
5.270
5.264
5.251
5.245
5.175
5.163
5.150
5.135
5.130
5.117
5.111
5.091
5.084
5.073
5.059
5.052
4.994
4.989
4.975
4.960
4.955
3.869
1.461
1.447
1.324
1.295
BocN
O
CO2Me
O
O
+
Boc
N
O
CO2Me
O
O
9
357
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
2.57
0.31
2.16
1.00
0.33
3.91
12.22
3.90
3.93
170.99
157.00
130.97
129.93
128.74
112.75
82.71
82.27
74.80
74.38
72.70
71.35
55.23
53.48
53.13
28.07
26.23
26.05
180
160
140
120
100
80
60
40
20
ppm


6.895
6.890
6.878
6.872
6.862
6.856
6.845
6.182
6.148
5.424
5.392
4.842
4.430
3.826
1.439
1.345
O
CO2Me
O
O
NHBoc
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
1.00
0.98
0.86
0.90
0.98
3.12
12.46
3.21
191.29
168.64
154.72
145.75
127.45
111.21
81.98
80.58
79.44
77.48
77.25
77.06
76.63
53.55
45.41
28.30
27.12
25.59
180
160
140
120
100
80
60
40
20
ppm

360


6.883
6.872
6.849
6.838
6.176
6.171
6.142
6.137
5.232
5.199
4.860
4.832
4.741
3.816
1.480
1.459
1.439
BocHN
CO2Me
O
O
O
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
1.02
1.00
0.92
0.94
1.02
3.00
3.04
3.25
8.85
192.17
170.24
154.88
148.77
128.04
112.67
86.22
80.53
53.25
50.50
28.21
27.29
26.19
180
160
140
120
100
80
60
40
20
ppm

361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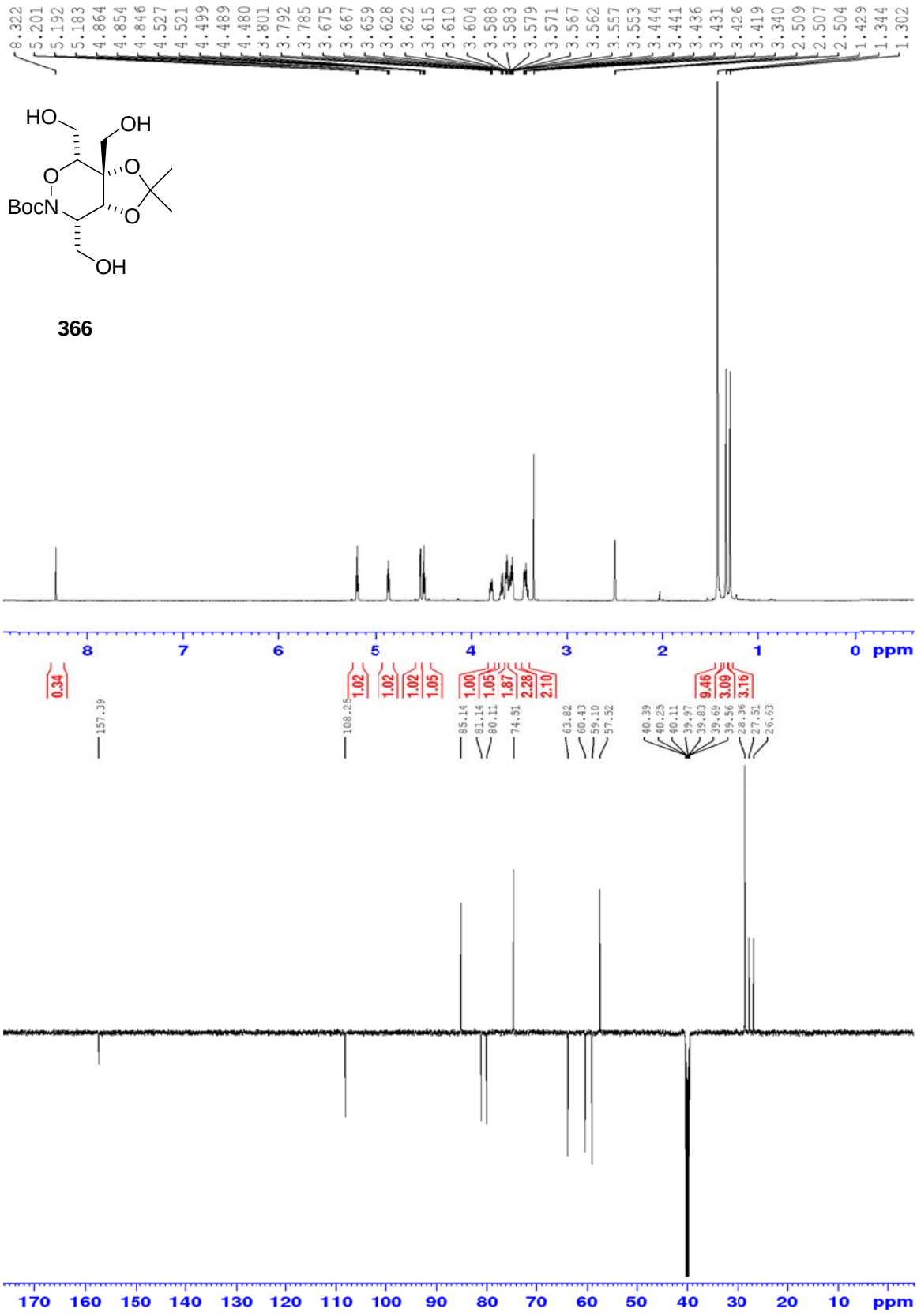
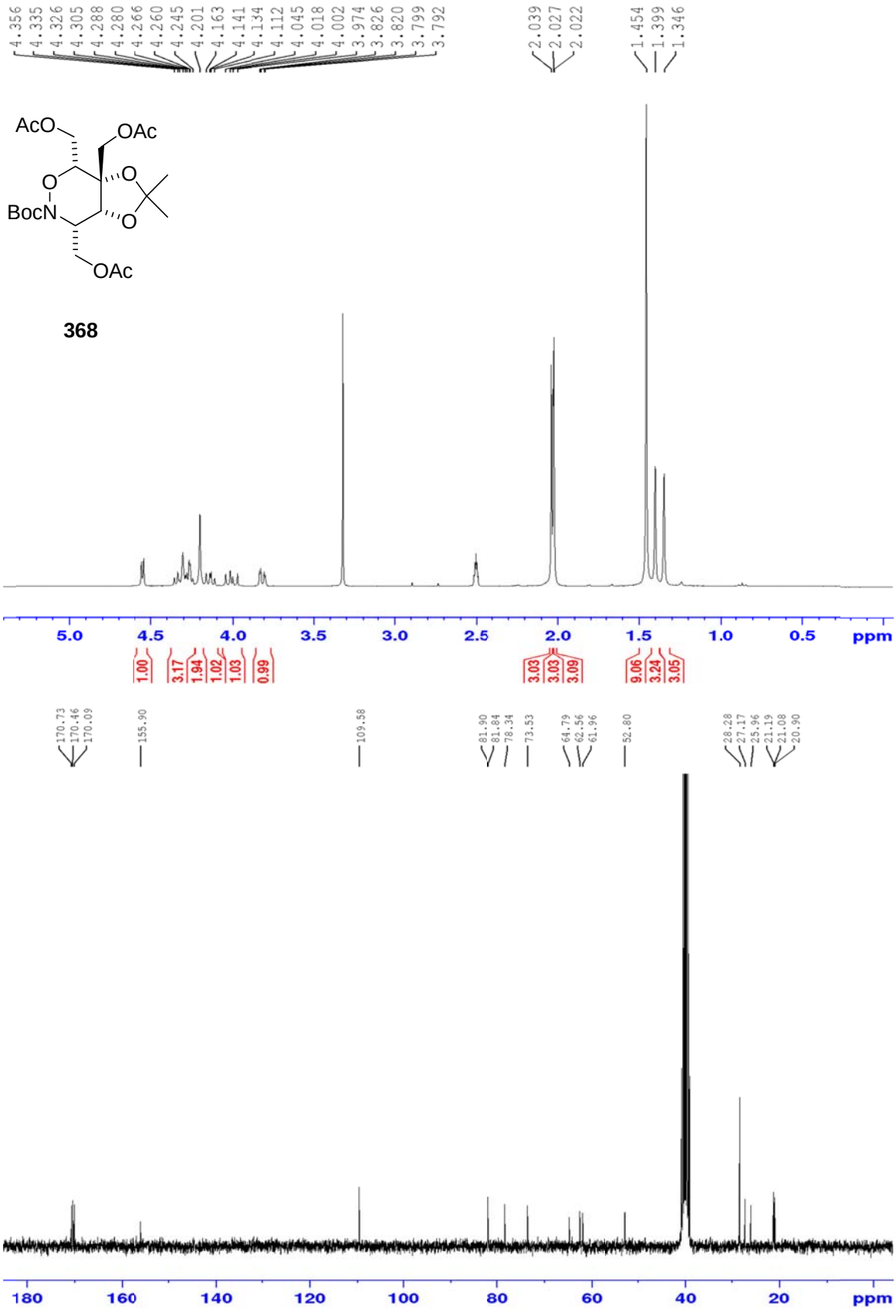
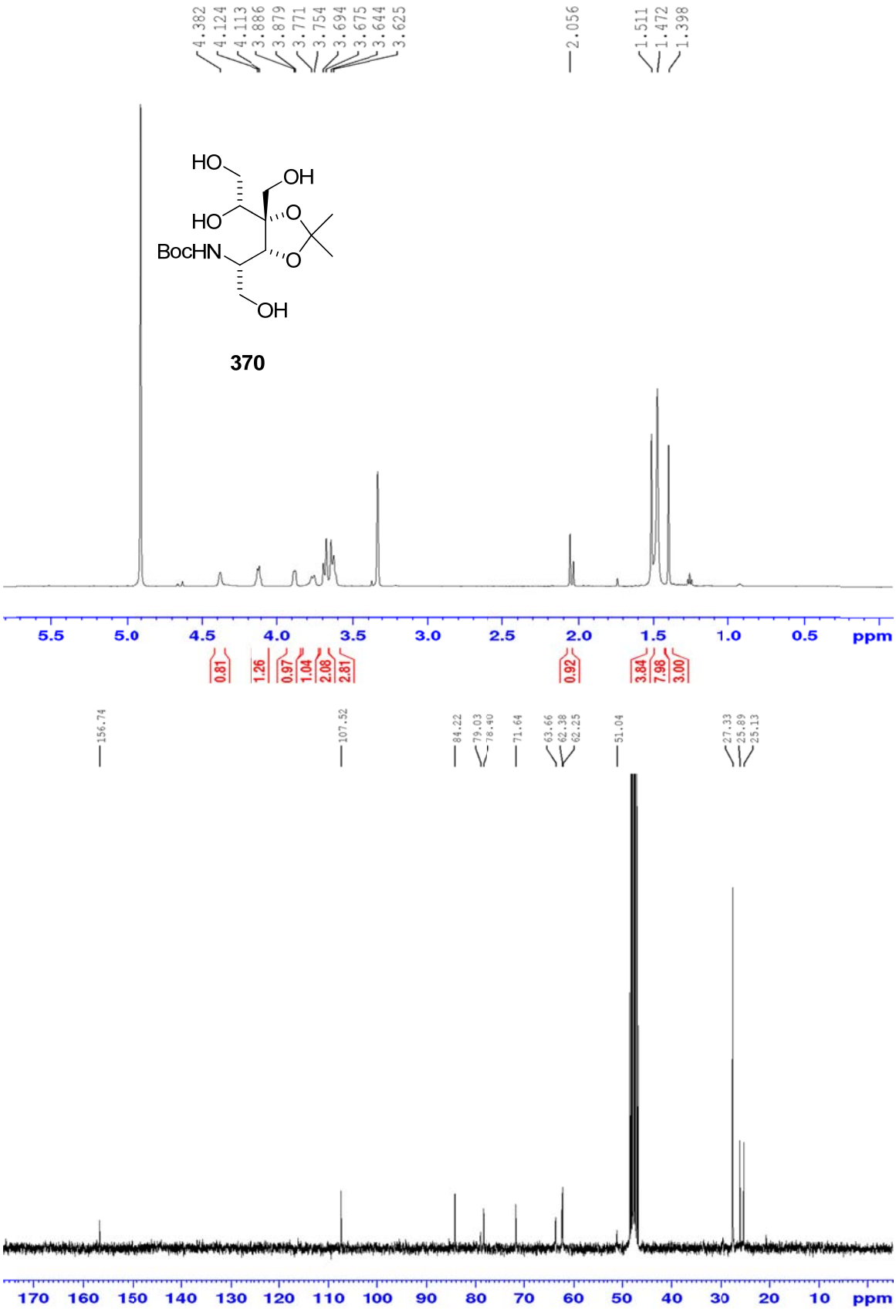
6.545
6.541
5.500
5.460
5.059
5.040
5.030
4.463
3.888
2.247
1.490
1.481
1.448
O
CO2Me
AcO
O
O
NHBoc
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
1.00
0.91
0.99
1.02
3.13
3.11
12.15
3.54
185.23
168.05
154.62
143.94
131.53
111.92
83.36
80.74
79.52
53.75
44.90
28.30
26.96
25.64
22.32
14.04
180
160
140
120
100
80
60
40
20
ppm

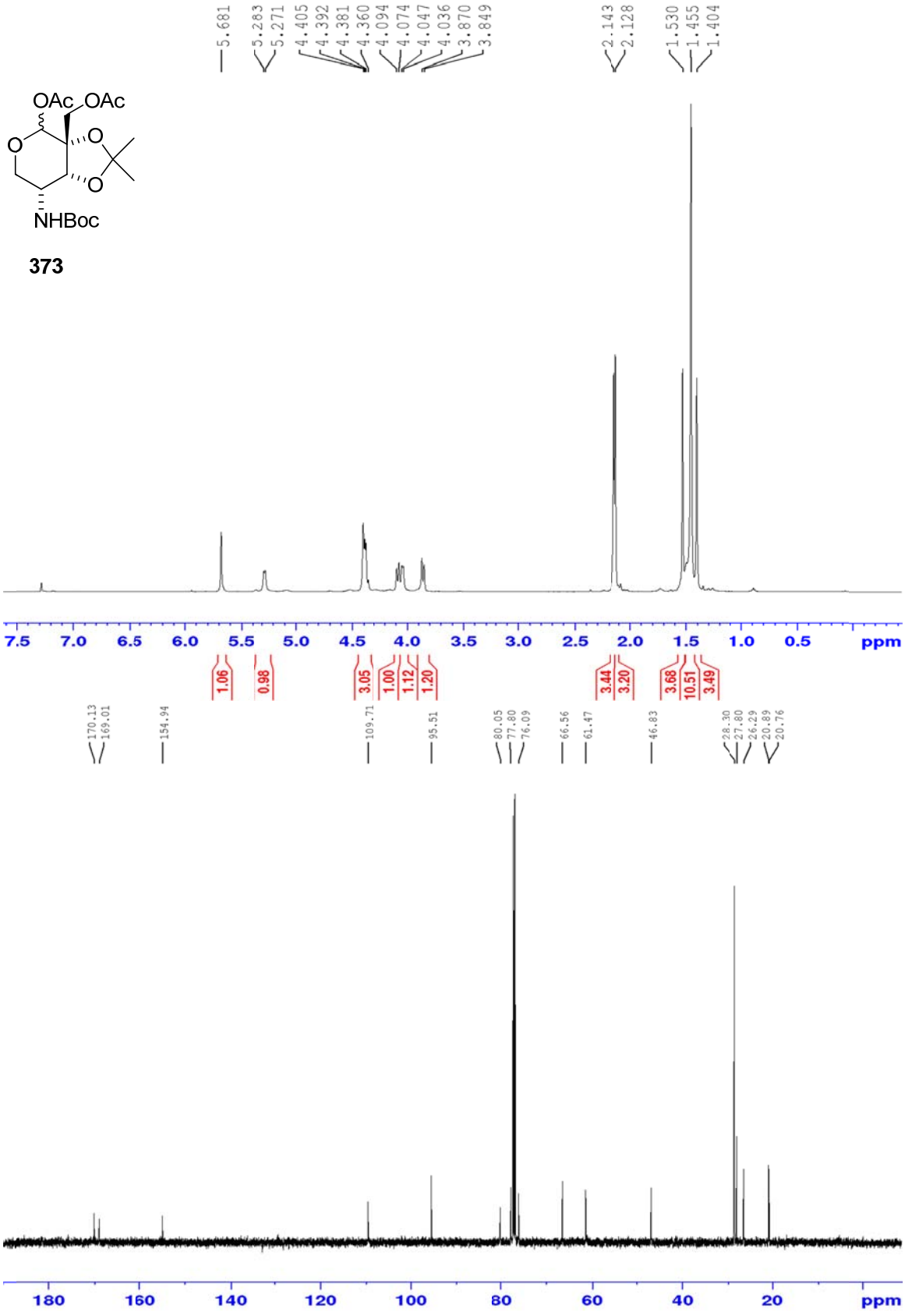
365


8.322
5.201
5.192
5.183
4.864
4.854
4.846
4.527
4.521
4.499
4.489
4.480
3.801
3.792
3.785
3.675
3.667
3.659
3.628
3.622
3.615
3.610
3.604
3.588
3.583
3.579
3.571
3.567
3.562
3.557
3.553
3.444
3.441
3.436
3.431
3.426
3.419
3.340
2.509
2.507
2.504
1.429
1.344
1.302
HO
OH
O
O
BocN
O
OH
8
7
6
5
4
3
2
1
0 ppm
0.34
1.02
1.02
1.02
1.05
1.00
1.05
1.87
2.28
2.10
9.46
3.09
3.16
157.39
108.25
85.14
81.14
80.11
74.51
63.82
60.43
59.10
57.52
40.39
40.25
40.11
39.97
39.83
39.69
39.56
28.36
27.51
26.63
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

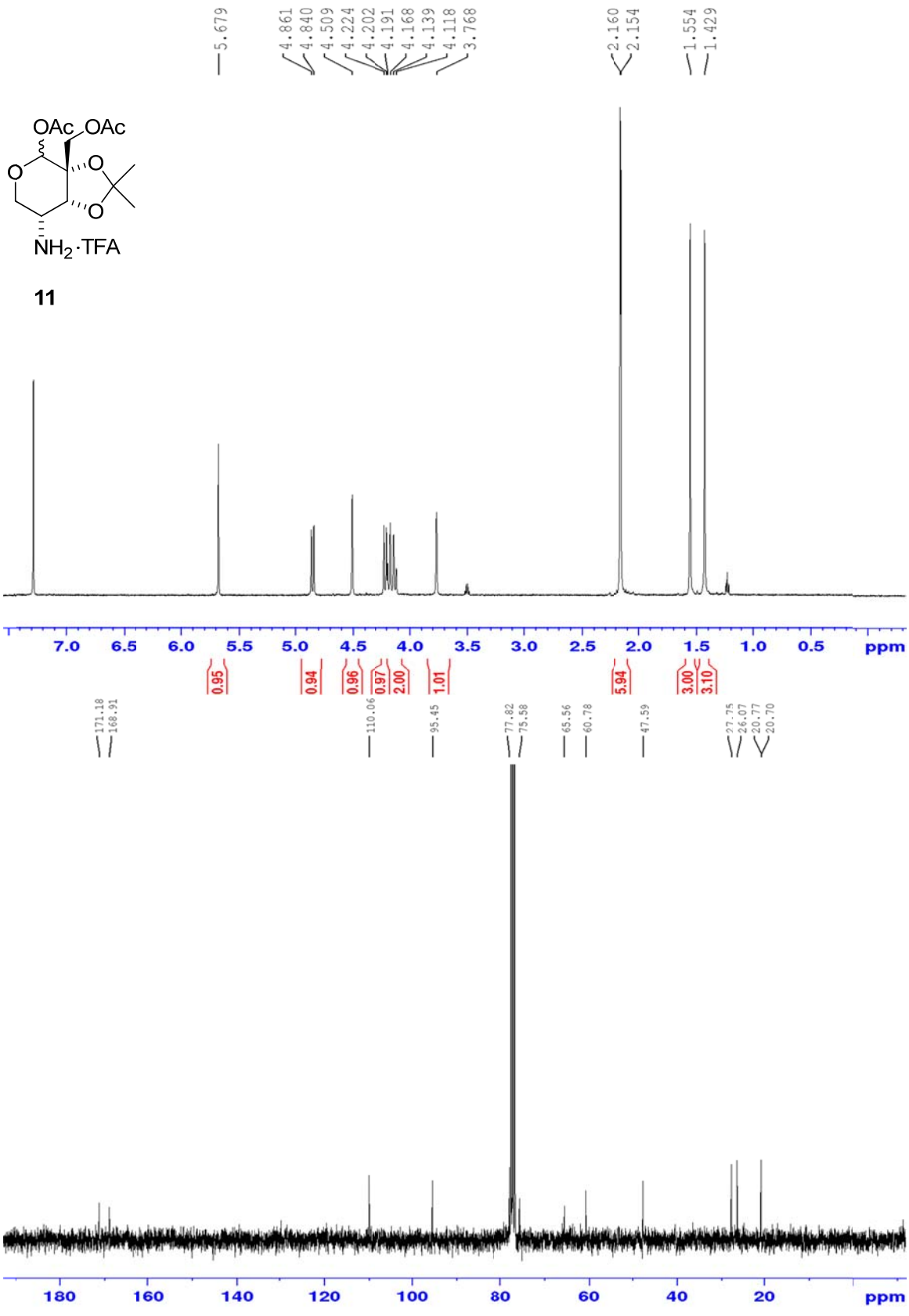
366

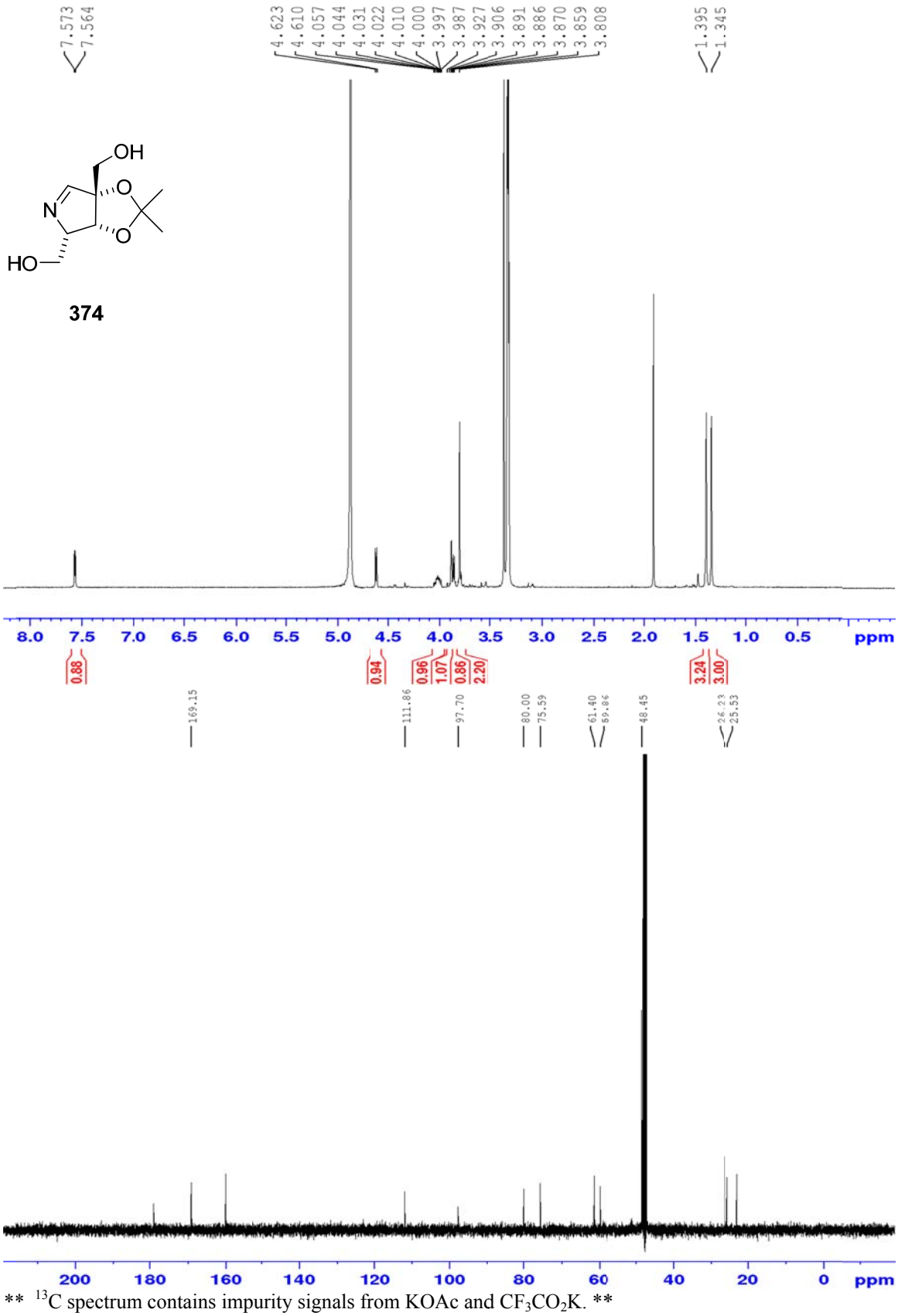

4.356
4.335
4.326
4.305
4.288
4.280
4.266
4.260
4.245
4.201
4.163
4.141
4.134
4.112
4.045
4.018
4.002
3.974
3.826
3.820
3.799
3.792
2.039
2.027
2.022
1.454
1.399
1.346
AcO
OAc
O
O
BocN
O
OAc
368
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
1.00
3.17
1.94
1.02
1.03
0.99
3.03
3.03
3.09
9.06
3.24
3.05
170.73
170.46
170.09
155.90
109.58
81.90
81.84
78.34
73.53
64.79
62.56
61.96
52.80
28.28
27.17
25.96
21.19
21.08
20.90
180
160
140
120
100
80
60
40
20
ppm


4.382
4.124
4.113
3.886
3.879
3.771
3.754
3.694
3.675
3.644
3.625
2.056
1.511
1.472
1.398
HO
OH
HO
O
BocHN
O
OH
370
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
0.81
1.26
0.97
1.04
2.08
2.81
0.92
3.84
7.98
3.00
156.74
107.52
84.22
79.03
78.40
71.64
63.66
62.38
62.25
51.04
27.33
25.89
25.13
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm


OAc
OAc
O
O
O
NHBoc
5.681
5.283
5.271
4.405
4.392
4.381
4.360
4.094
4.074
4.047
4.036
3.870
3.849
2.143
2.128
1.530
1.455
1.404
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
1.06
0.98
3.05
1.00
1.12
1.20
3.44
3.20
3.68
10.51
3.49
170.13
169.01
154.94
109.71
95.51
80.05
77.80
76.09
66.56
61.47
46.83
28.30
27.80
26.29
20.89
20.76
180
160
140
120
100
80
60
40
20
ppm

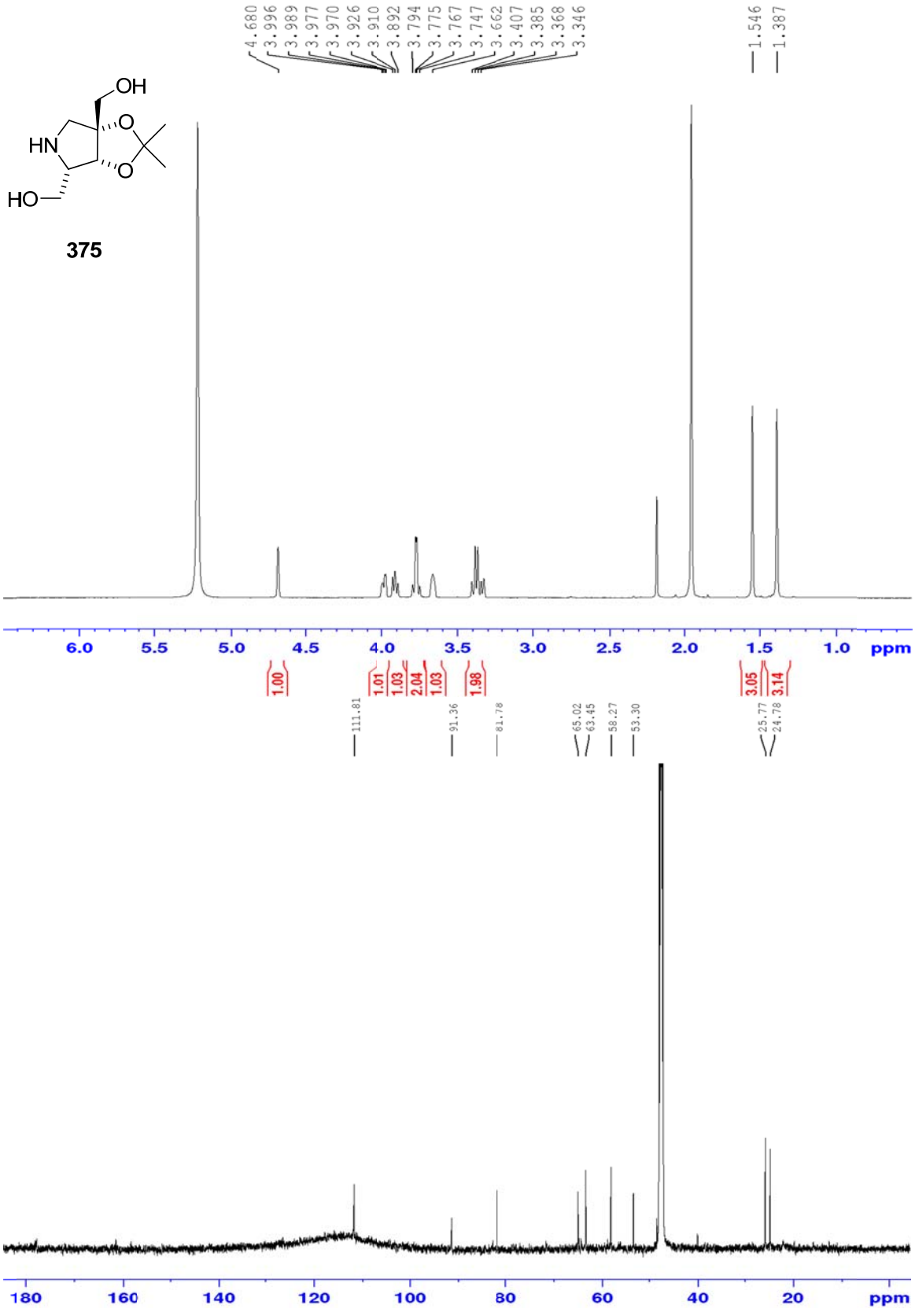
373

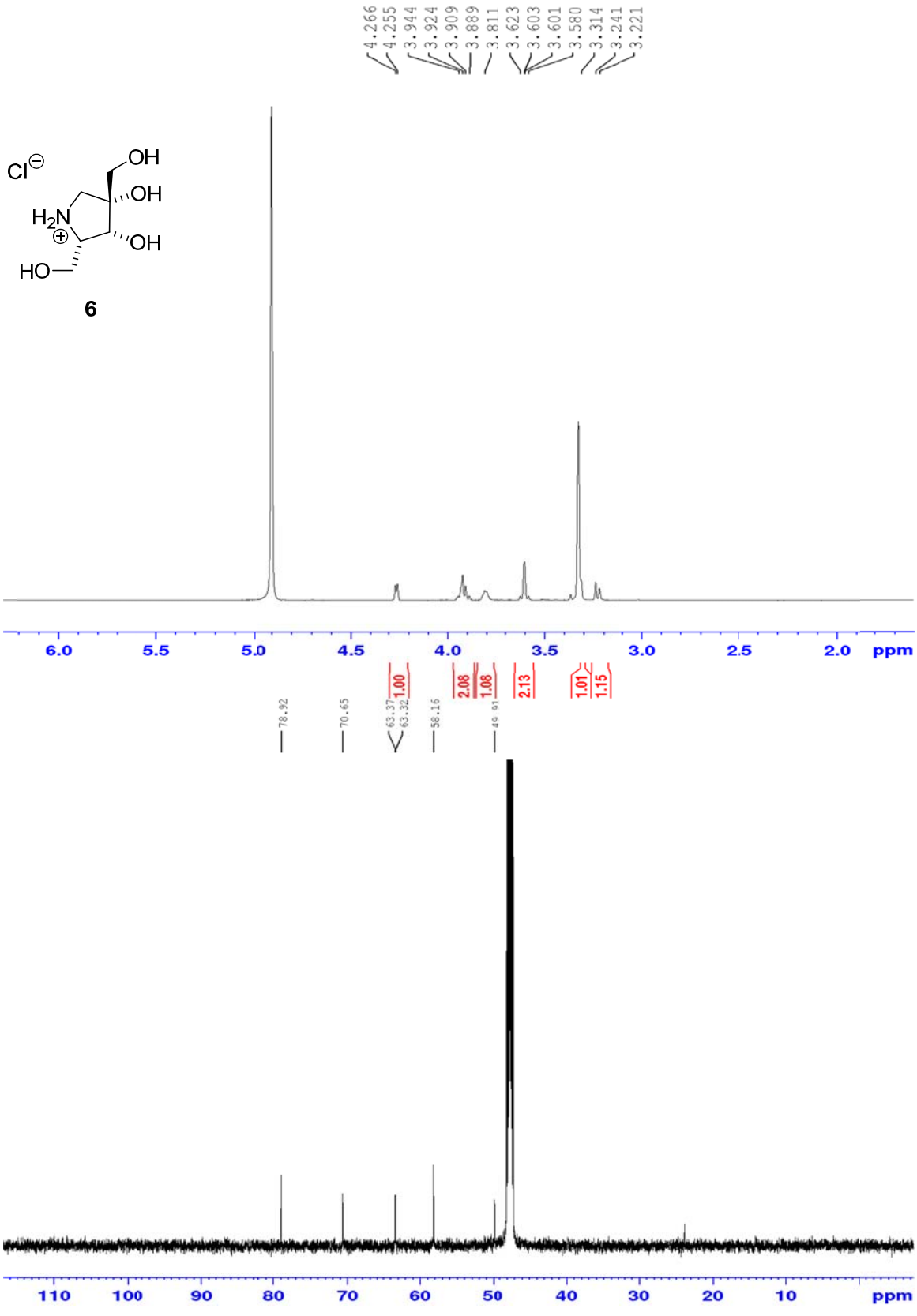

5.679
4.861
4.840
4.509
4.224
4.202
4.191
4.168
4.139
4.118
3.768
2.160
2.154
1.554
1.429
OAc
OAc
O
O
O
NH2·TFA
11
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
0.95
0.94
0.96
0.97
2.00
1.01
5.94
3.00
3.10
171.18
168.91
110.06
95.45
77.82
75.58
65.56
60.78
47.59
27.75
26.07
20.77
20.70
180
160
140
120
100
80
60
40
20
ppm

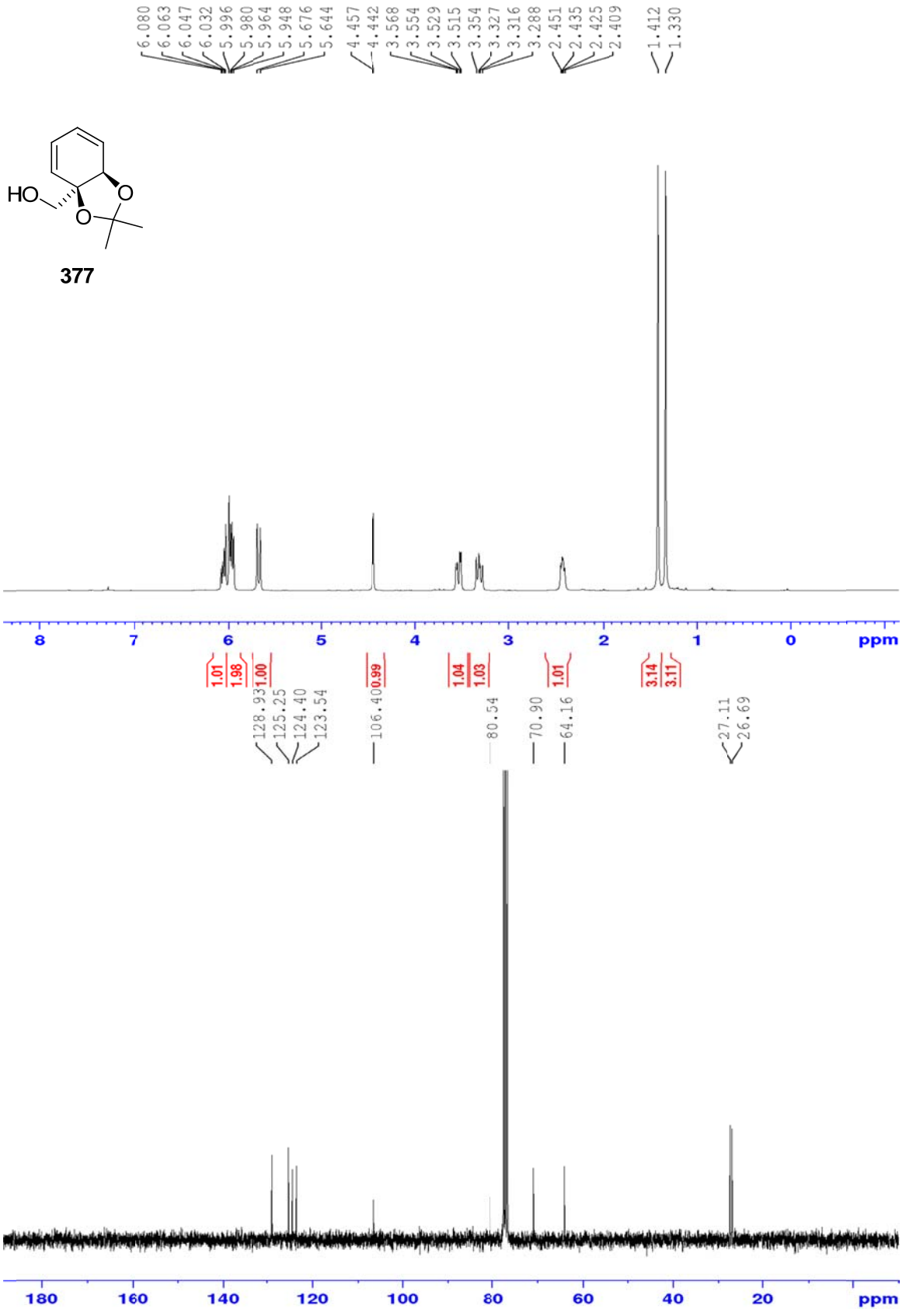


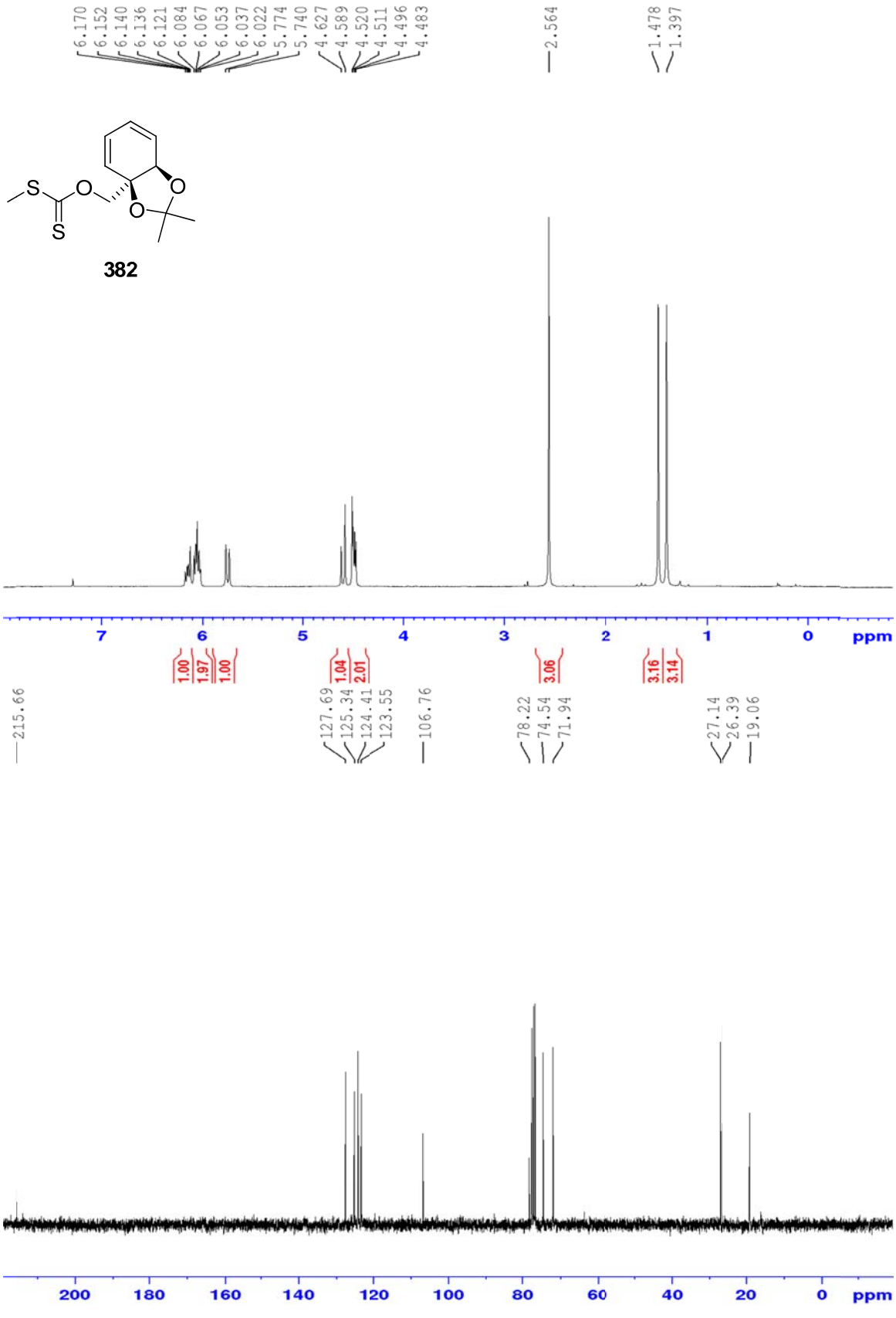
374

** ^{13}C spectrum contains impurity signals from KOAc and CF_3CO_2K. **

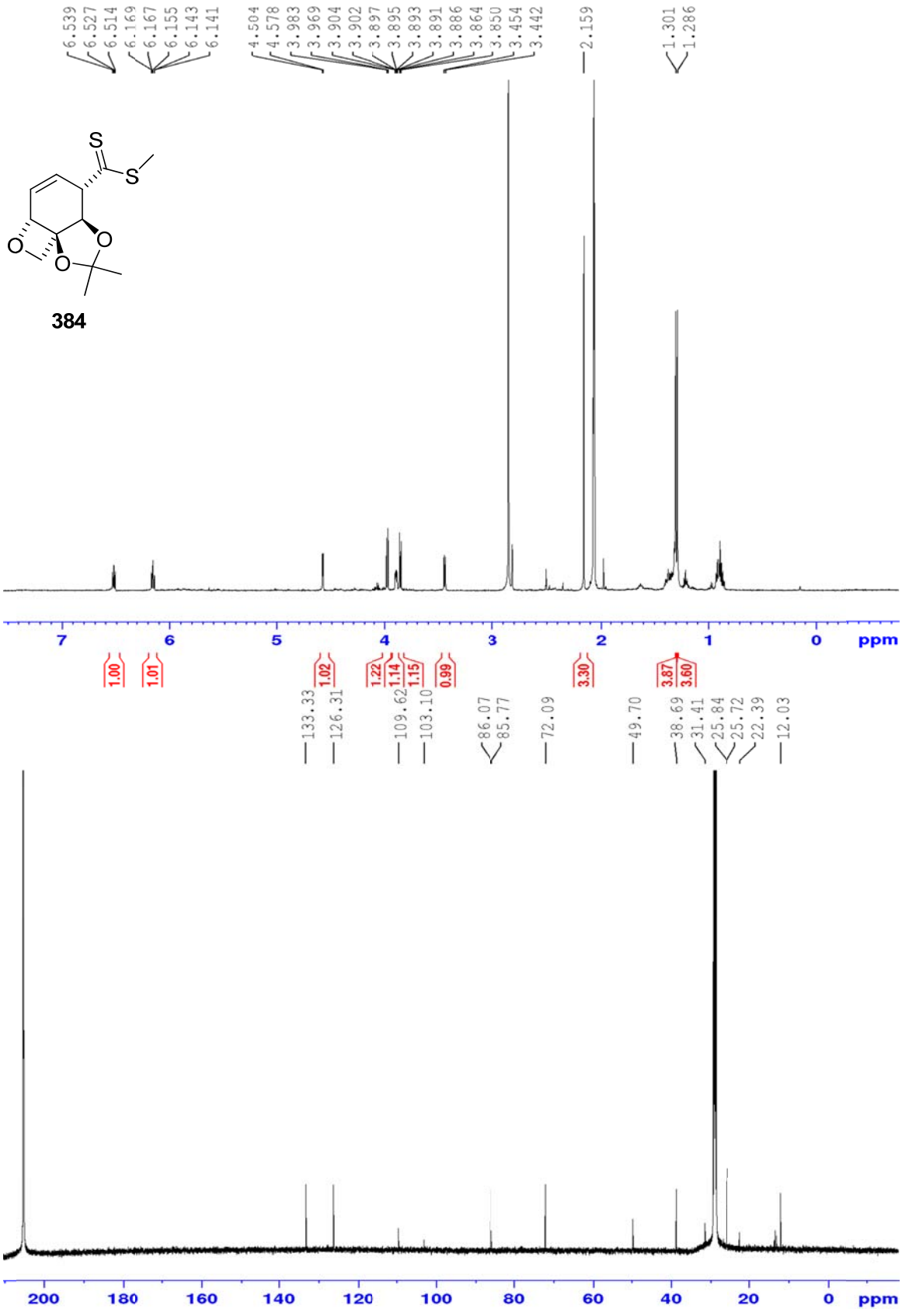

4.680
3.996
3.989
3.977
3.970
3.926
3.910
3.892
3.794
3.775
3.767
3.747
3.662
3.407
3.385
3.368
3.346
1.546
1.387
OH
HN
O
O
HO
375
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
1.00
1.01
1.03
2.04
1.03
1.98
3.05
3.14
111.81
91.36
81.78
65.02
63.45
58.27
53.30
25.77
24.78
180
160
140
120
100
80
60
40
20
ppm

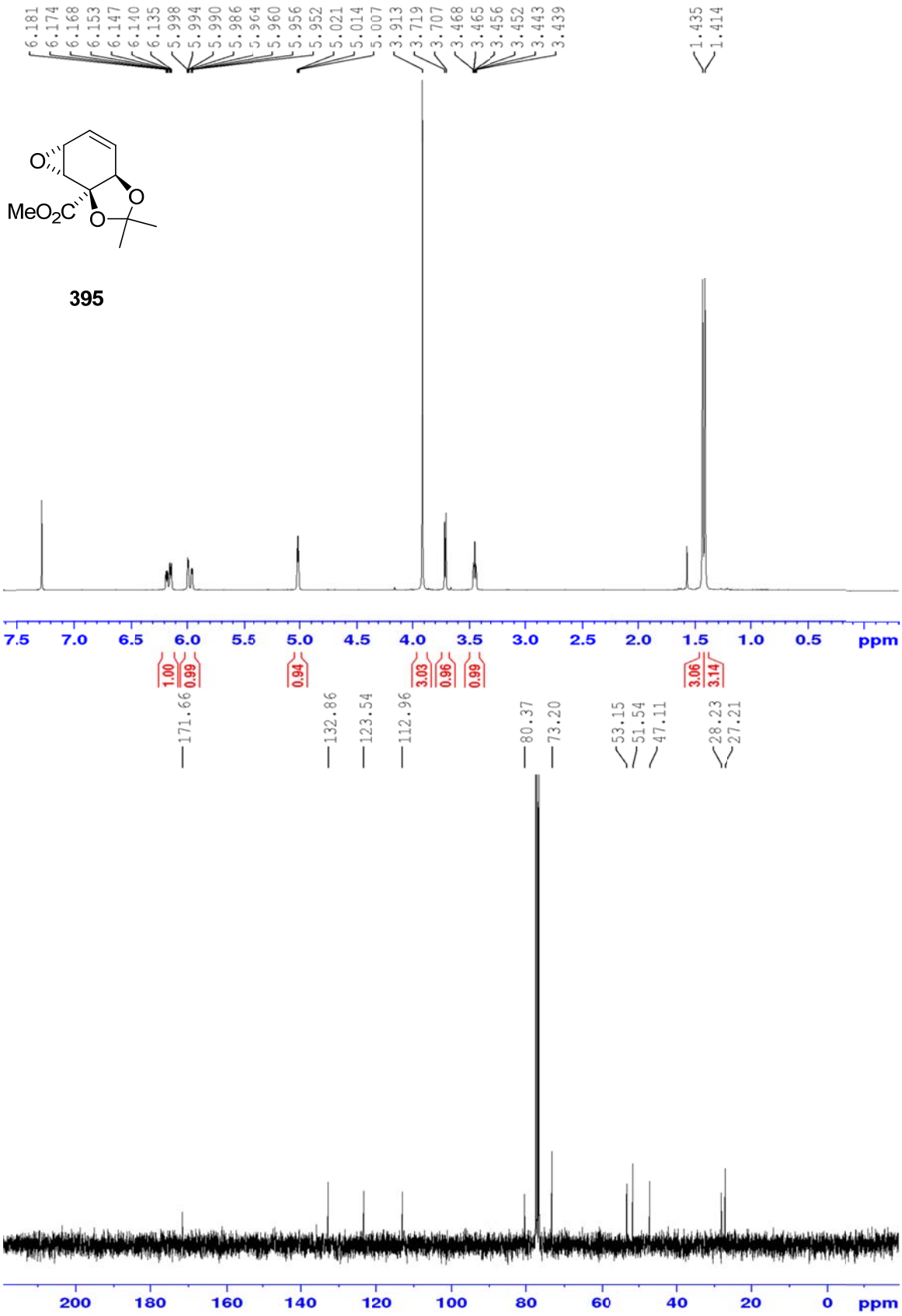

4.266
4.255
3.944
3.924
3.909
3.889
3.811
3.623
3.603
3.601
3.580
3.314
3.241
3.221
Cl⊖
OH
OH
H2N⊕
OH
HO
6
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
ppm
1.00
2.08
1.08
2.13
1.01
1.15
78.92
70.65
63.37
63.32
58.16
49.91
110
100
90
80
70
60
50
40
30
20
10
ppm

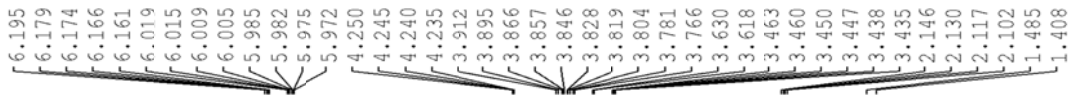

6.080
6.063
6.047
6.032
5.996
5.980
5.964
5.948
5.676
5.644
4.457
4.442
3.568
3.554
3.529
3.515
3.354
3.327
3.316
3.288
2.451
2.435
2.425
2.409
1.412
1.330
HO
O
O
377
8
7
6
5
4
3
2
1
0
ppm
1.01
1.98
1.00
0.99
1.04
1.03
1.01
3.14
3.11
128.93
125.25
124.40
123.54
106.40
80.54
70.90
64.16
27.11
26.69
180
160
140
120
100
80
60
40
20
ppm


6.170
6.152
6.140
6.136
6.121
6.084
6.067
6.053
6.037
6.022
5.774
5.740
4.627
4.589
4.520
4.511
4.496
4.483
2.564
1.478
1.397
S
O
S
O
O
7
6
5
4
3
2
1
0
ppm
1.00
1.97
1.00
1.04
2.01
3.06
3.16
3.14
215.66
127.69
125.34
124.41
123.55
106.76
78.22
74.54
71.94
27.14
26.39
19.06
200
180
160
140
120
100
80
60
40
20
0
ppm

382


6.539
6.527
6.514
6.169
6.167
6.155
6.143
6.141
4.504
4.578
3.983
3.969
3.904
3.902
3.897
3.895
3.893
3.891
3.886
3.864
3.850
3.454
3.442
2.159
1.301
1.286
S
S
O
O
O
384
7
6
5
4
3
2
1
0
ppm
1.00
1.01
1.02
1.22
1.14
1.15
0.99
3.30
3.87
3.60
133.33
126.31
109.62
103.10
86.07
85.77
72.09
49.70
38.69
31.41
25.84
25.72
22.39
12.03
200
180
160
140
120
100
80
60
40
20
0
ppm


6.181
6.174
6.168
6.153
6.147
6.140
6.135
5.998
5.994
5.990
5.986
5.964
5.960
5.956
5.952
5.021
5.014
5.007
3.913
3.719
3.707
3.468
3.465
3.456
3.452
3.443
3.439
1.435
1.414
O
MeO2C
O
O
395
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
1.00
0.99
0.94
3.03
0.96
0.99
3.06
3.14
171.66
132.86
123.54
112.96
80.37
73.20
53.15
51.54
47.11
28.23
27.21
200
180
160
140
120
100
80
60
40
20
0
ppm


6.195
6.179
6.174
6.166
6.161
6.019
6.015
6.009
6.005
5.985
5.982
5.975
5.972
4.250
4.245
4.240
4.235
3.912
3.895
3.866
3.857
3.846
3.828
3.819
3.804
3.781
3.766
3.630
3.618
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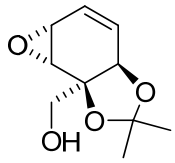
3.447
3.438
3.435
2.146
2.130
2.117
2.102
1.485
1.408


O
O
O
OH

406

8
7
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5
4
3
2
1
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ppm
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1.03
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2.25
0.97
1.01
0.99
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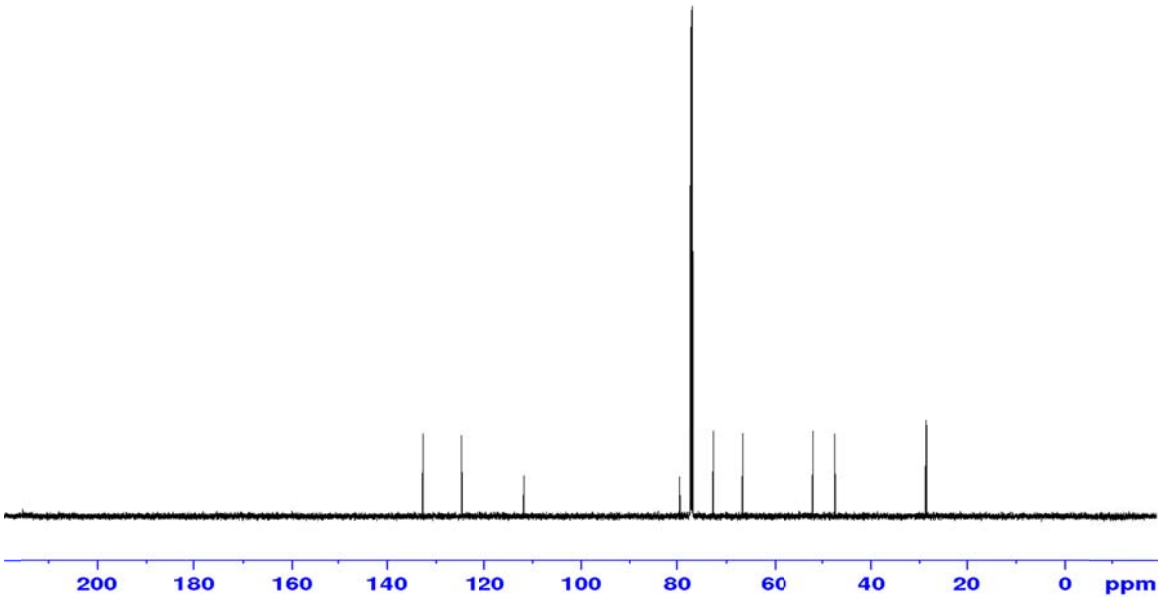
132.77
124.83
111.74
79.47
72.60
66.60
51.87
47.40
28.72
28.52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180
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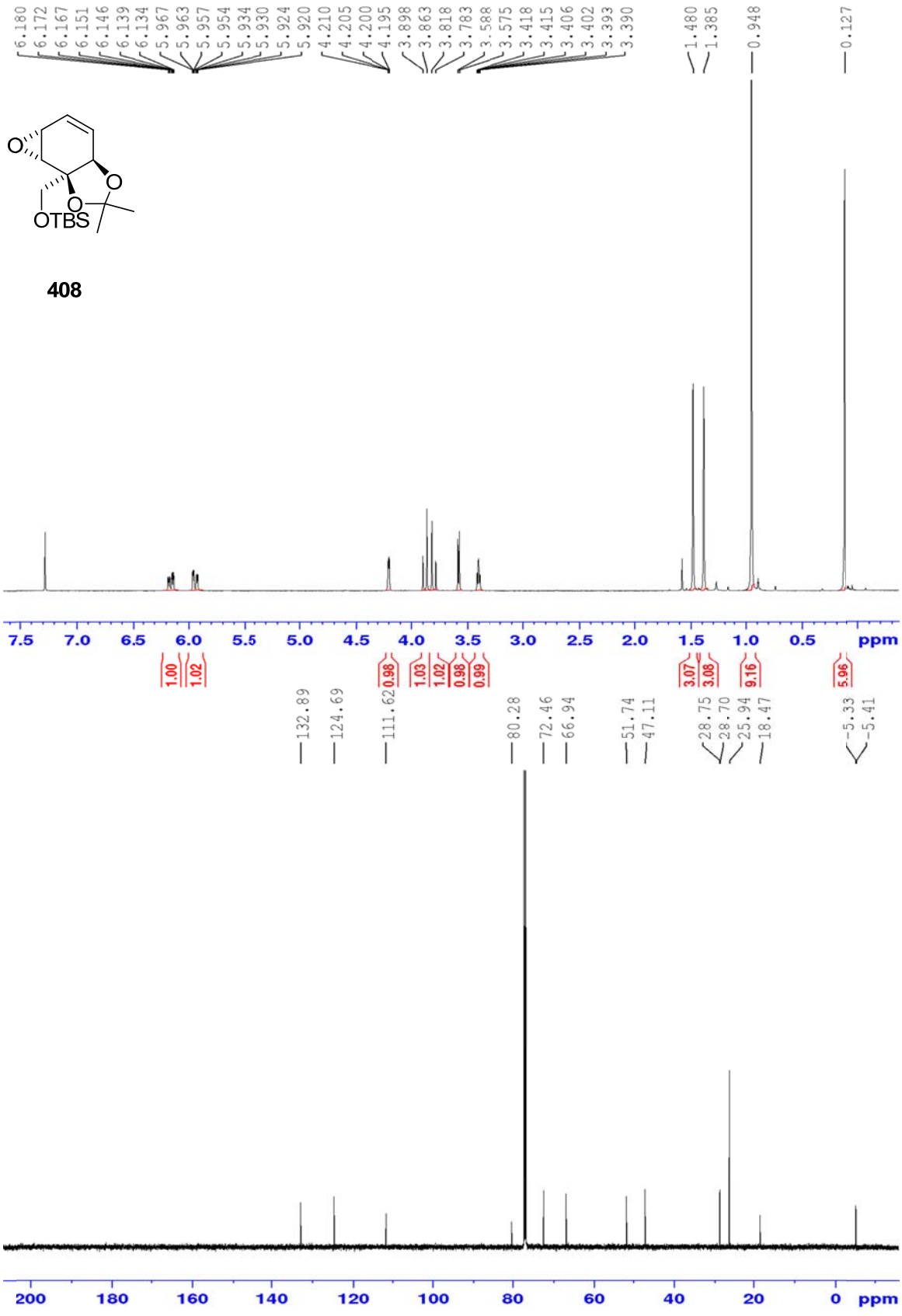
ppm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6.172
6.167
6.151
6.146
6.139
6.134
5.967
5.963
5.957
5.954
5.934
5.930
5.924
5.920
4.210
4.205
4.200
4.195
3.898
3.863
3.818
3.783
3.588
3.575
3.418
3.415
3.406
3.402
3.393
3.390
1.480
1.385
0.948
0.127
O
O
O
OTBS
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
1.00
1.02
0.98
1.03
1.02
0.98
0.99
3.07
3.08
9.16
5.96
132.89
124.69
111.62
80.28
72.46
66.94
51.74
47.11
28.75
28.70
25.94
18.47
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-5.41
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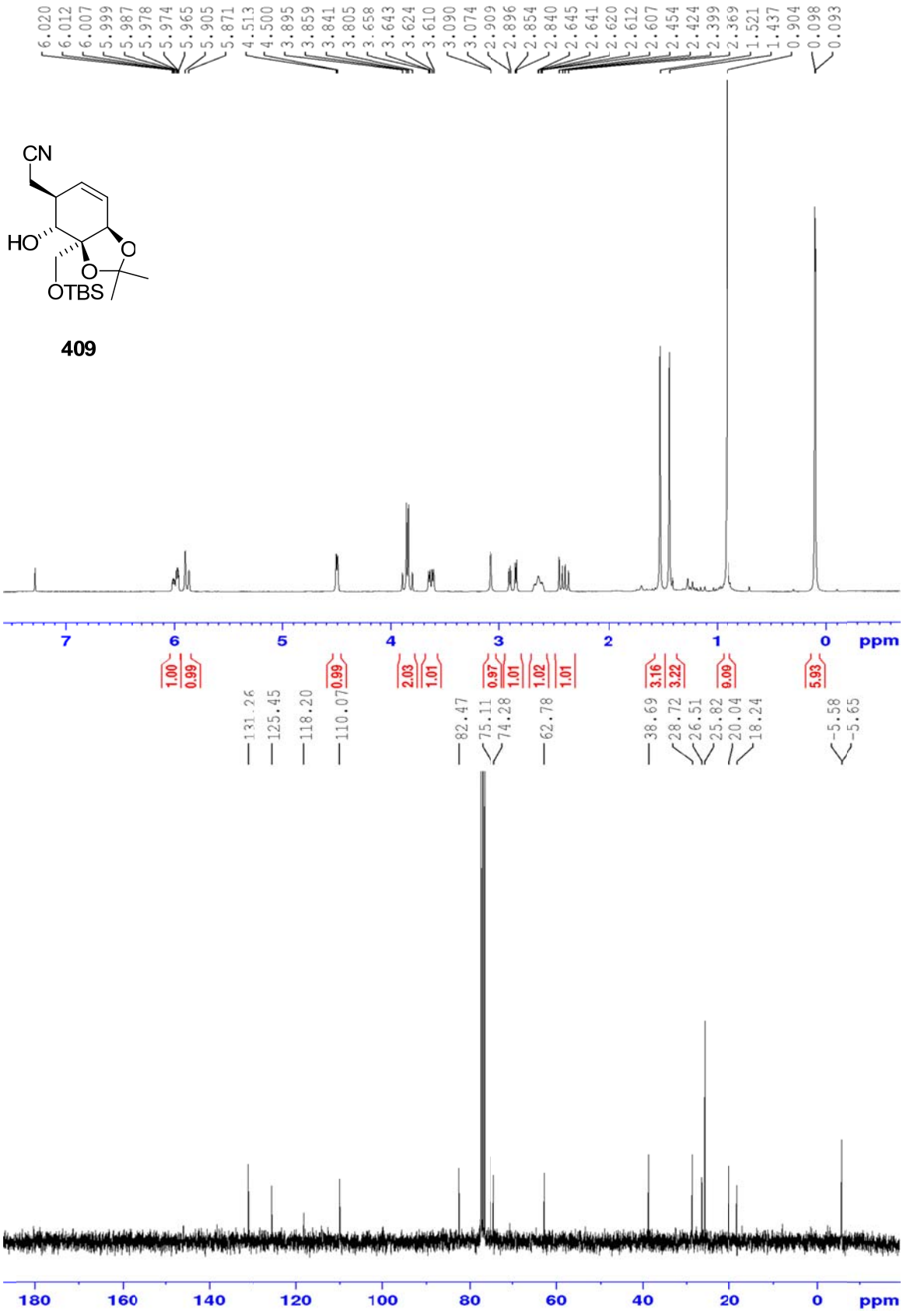
180
160
140
120
100
80
60
40
20
0
ppm

408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6.007
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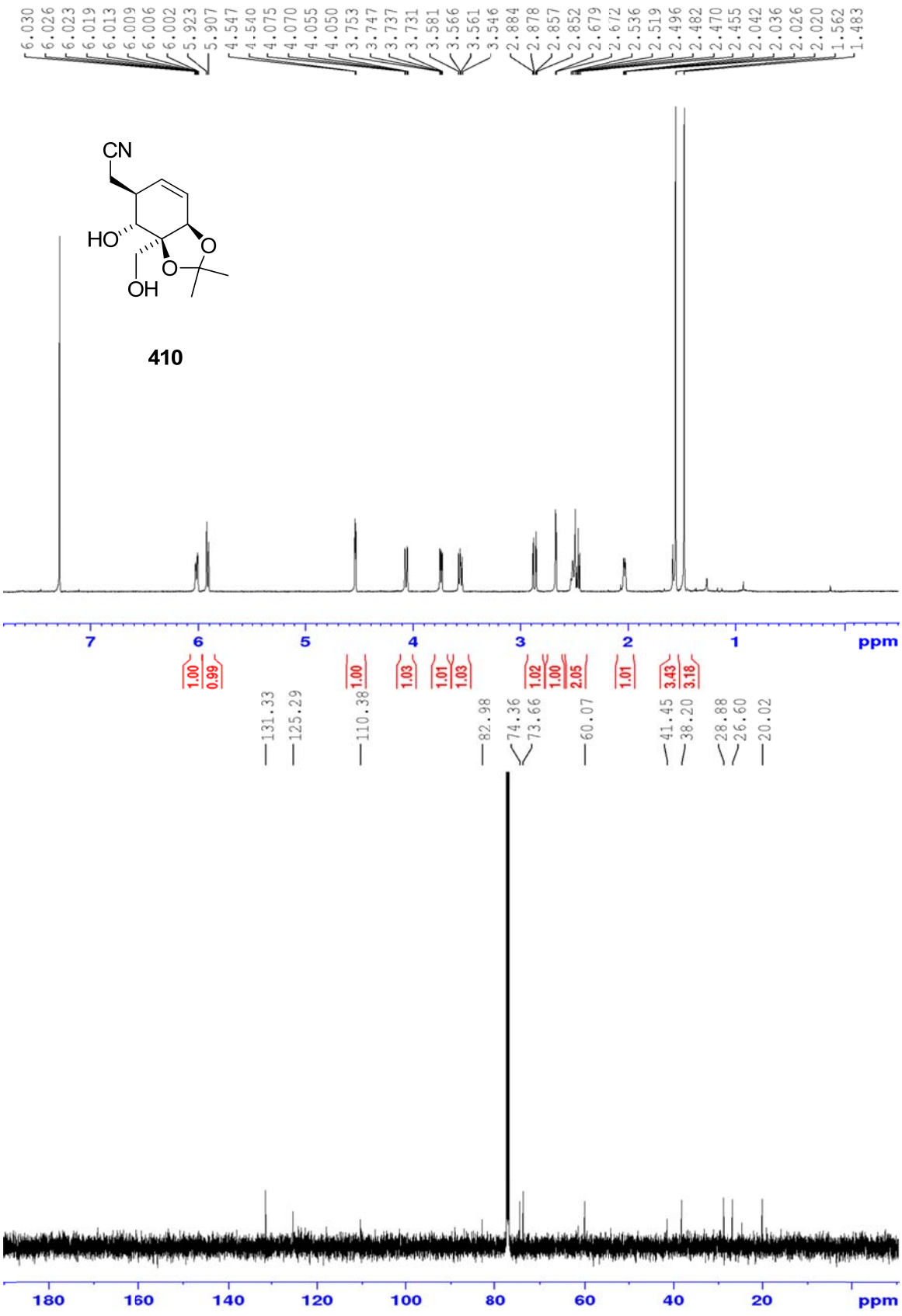
5.974
5.965
5.905
5.871
4.513
4.500
3.895
3.859
3.841
3.805
3.658
3.643
3.624
3.610
3.090
3.074
2.909
2.896
2.854
2.840
2.645
2.641
2.620
2.612
2.607
2.454
2.424
2.399
2.369
1.521
1.437
0.904
0.098
0.093
CN
HO
O
O
OTBS
409
7
6
5
4
3
2
1
0
ppm
1.00
0.99
0.99
2.03
1.01
0.97
1.01
1.02
1.01
3.16
3.22
9.00
5.93
131.26
125.45
118.20
110.07
82.47
75.11
74.28
62.78
38.69
28.72
26.51
25.82
20.04
18.24
-5.58
-5.65
180
160
140
120
100
80
60
40
20
0
ppm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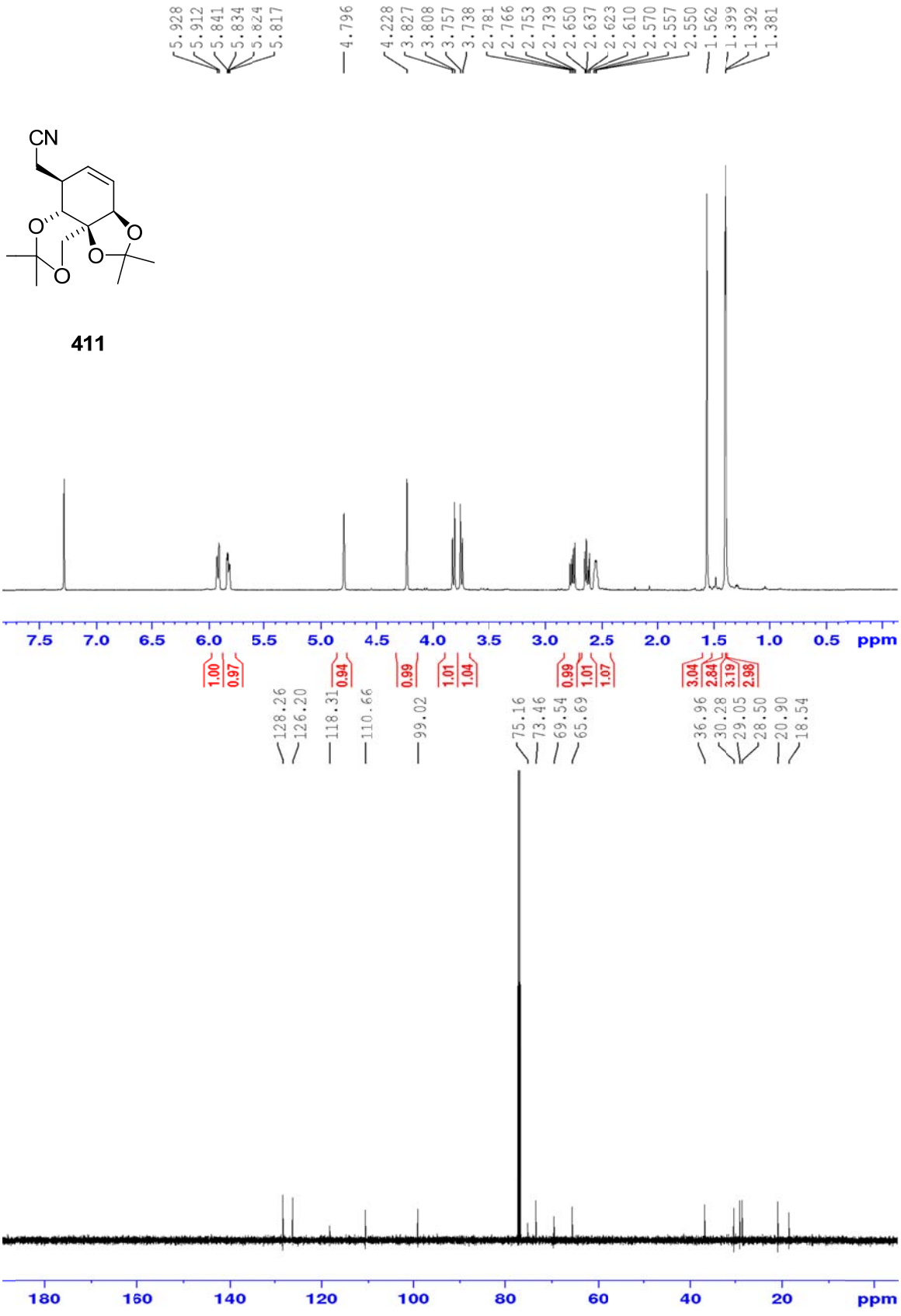
6.026
6.023
6.019
6.013
6.009
6.006
6.002
5.923
5.907
4.547
4.540
4.075
4.070
4.055
4.050
3.753
3.747
3.737
3.731
3.581
3.566
3.561
3.546
2.884
2.878
2.857
2.852
2.679
2.672
2.536
2.519
2.496
2.482
2.470
2.455
2.042
2.036
2.026
2.020
1.562
1.483
CN
HO
O
O
OH
410
7
6
5
4
3
2
1
ppm
1.00
0.99
1.00
1.03
1.01
1.03
1.02
1.00
2.05
1.01
3.43
3.18
131.33
125.29
110.38
82.98
74.36
73.66
60.07
41.45
38.20
28.88
26.60
20.02
180
160
140
120
100
80
60
40
20
ppm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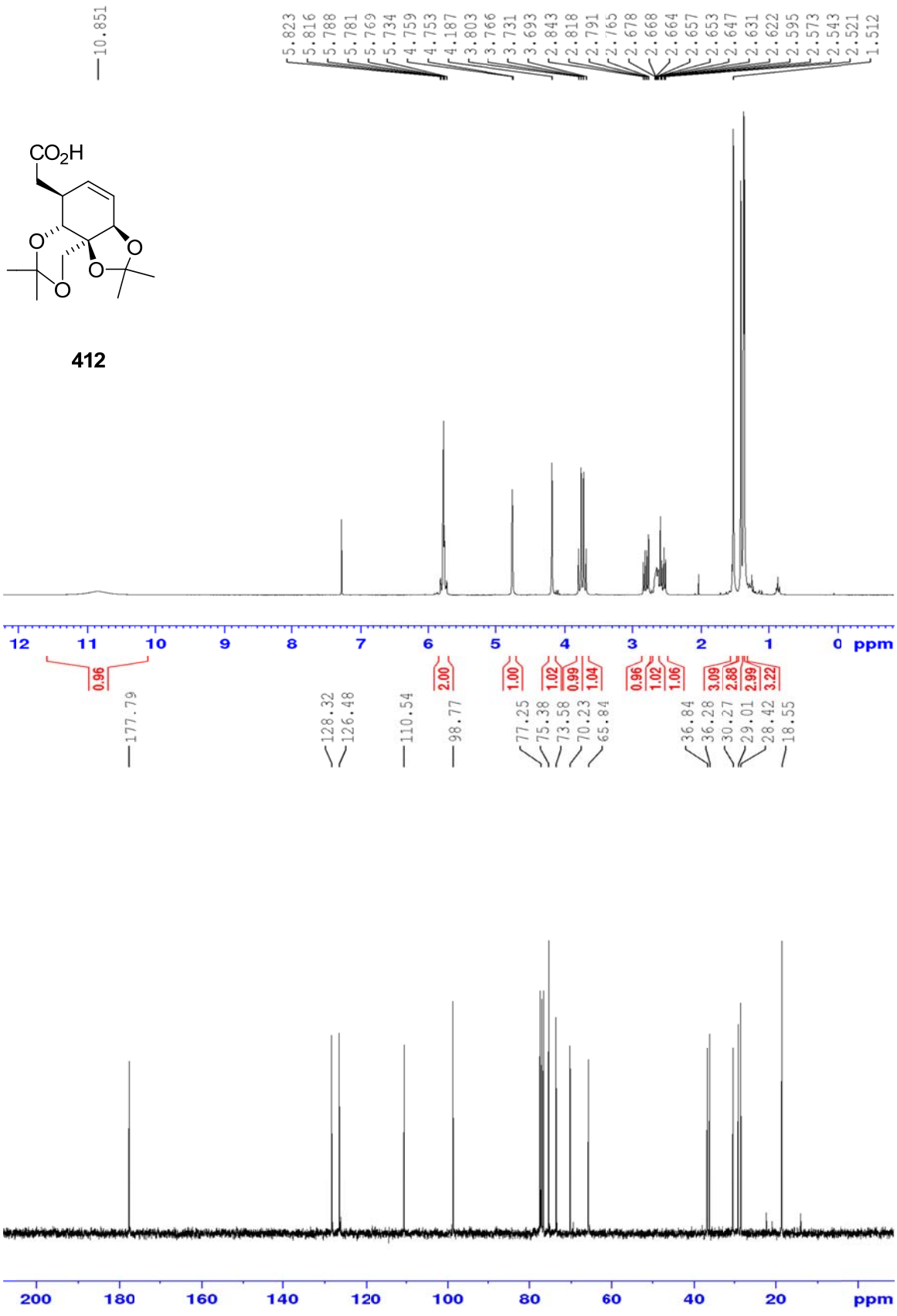
4.228
3.827
3.808
3.757
3.738
2.781
2.766
2.753
2.739
2.650
2.637
2.623
2.610
2.570
2.557
2.550
1.562
1.399
1.392
1.381
CN
O
O
O
O
411
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
1.00
0.97
0.94
0.99
1.01
1.04
0.99
1.01
1.07
3.04
2.84
3.19
2.98
128.26
126.20
118.31
110.66
99.02
75.16
73.46
69.54
65.69
36.96
30.28
29.05
28.50
20.90
18.54
180
160
140
120
100
80
60
40
20
ppm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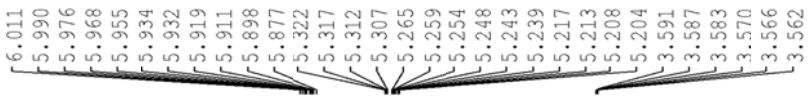
5.823
5.816
5.788
5.781
5.769
5.734
4.759
4.753
4.187
3.803
3.766
3.731
3.693
2.843
2.818
2.791
2.765
2.678
2.668
2.664
2.657
2.653
2.647
2.631
2.622
2.595
2.573
2.543
2.521
1.512
CO2H
O
O
O
O
412
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm
0.96
2.00
1.00
1.02
0.99
1.04
0.96
1.02
1.06
3.09
2.88
2.99
3.22
177.79
128.32
126.48
110.54
98.77
77.25
75.38
73.58
70.23
65.84
36.84
36.28
30.27
29.01
28.42
18.55
200
180
160
140
120
100
80
60
40
20
ppm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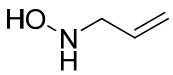
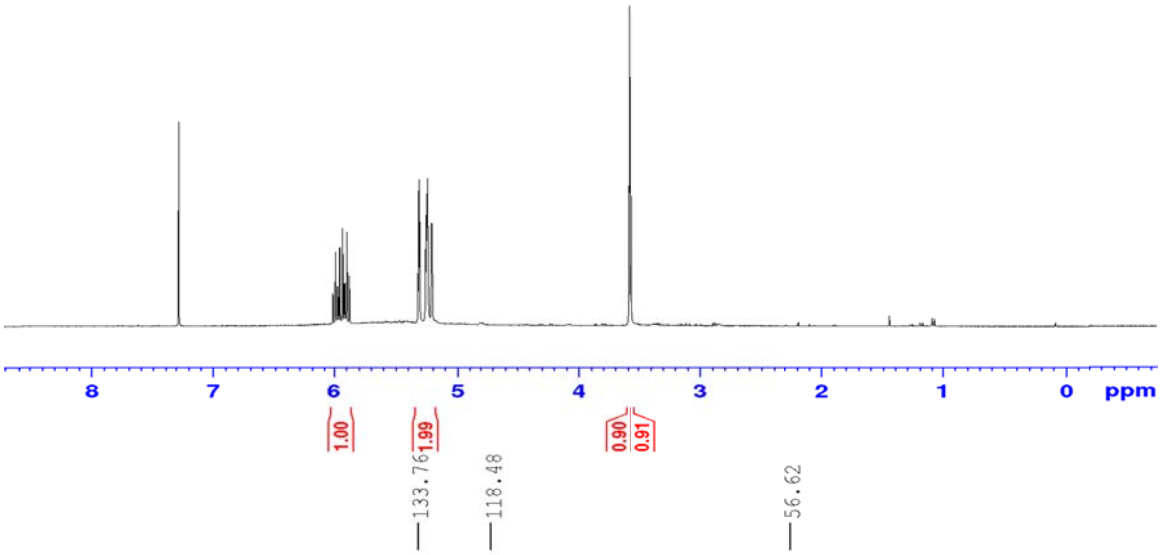
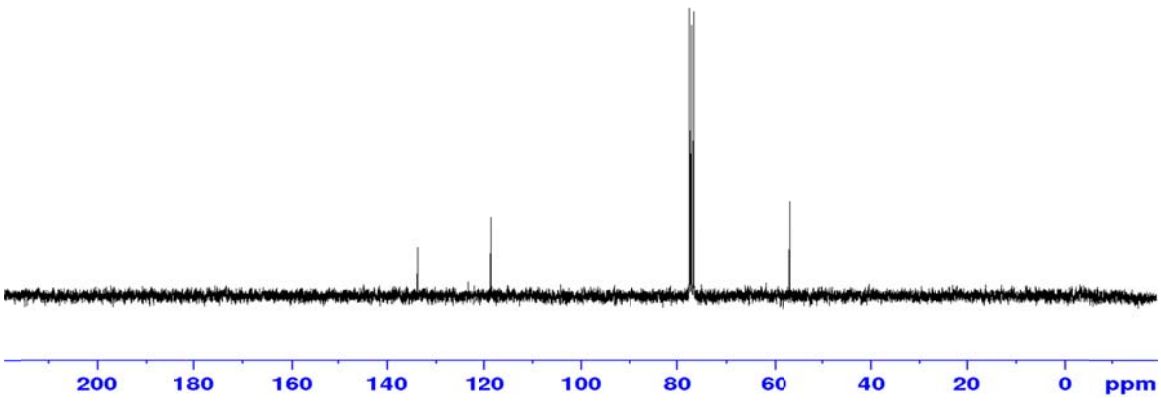
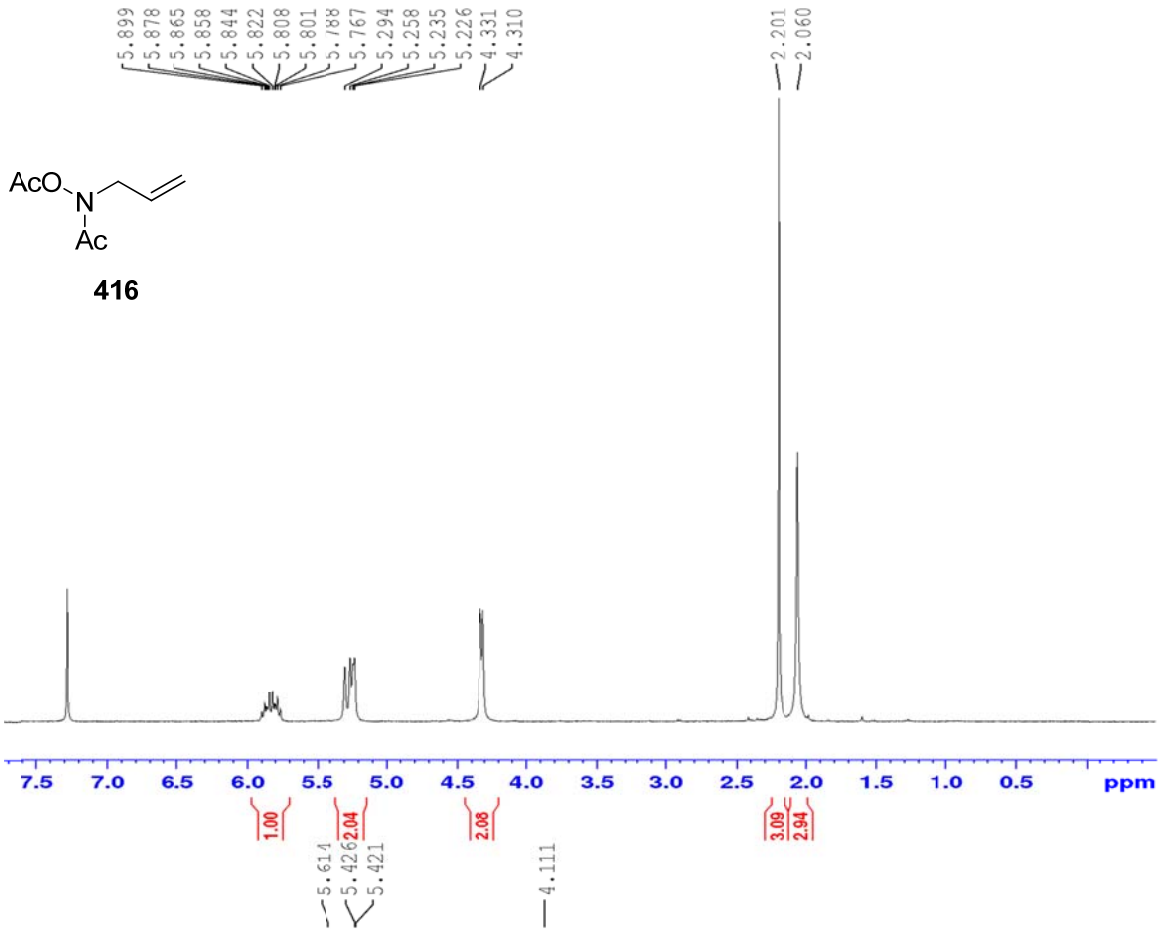
5.990
5.976
5.968
5.955
5.934
5.932
5.919
5.911
5.898
5.877
5.322
5.317
5.312
5.307
5.265
5.259
5.254
5.248
5.243
5.239
5.217
5.213
5.208
5.204
3.591
3.587
3.583
3.570
3.566
3.562


HO
N
H

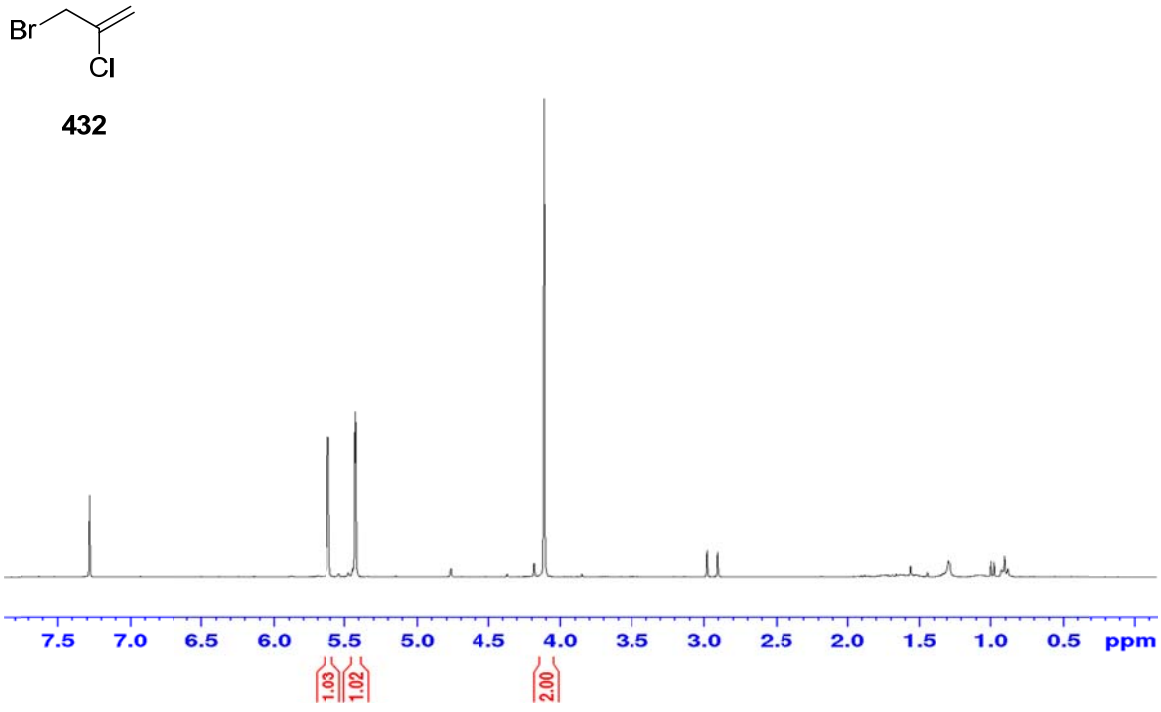
413


8
7
6
5
4
3
2
1
0
ppm
1.00
1.99
0.90
0.91
133.76
118.48
56.62


200
180
160
140
120
100
80
60
40
20
0
ppm


5.899
5.878
5.865
5.858
5.844
5.822
5.808
5.801
5.767
5.294
5.258
5.235
5.226
4.331
4.310
2.201
2.060
AcO
N
Ac
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
1.00
2.04
2.08
3.09
2.94
5.614
5.426
5.421
4.111

416


Br
Cl
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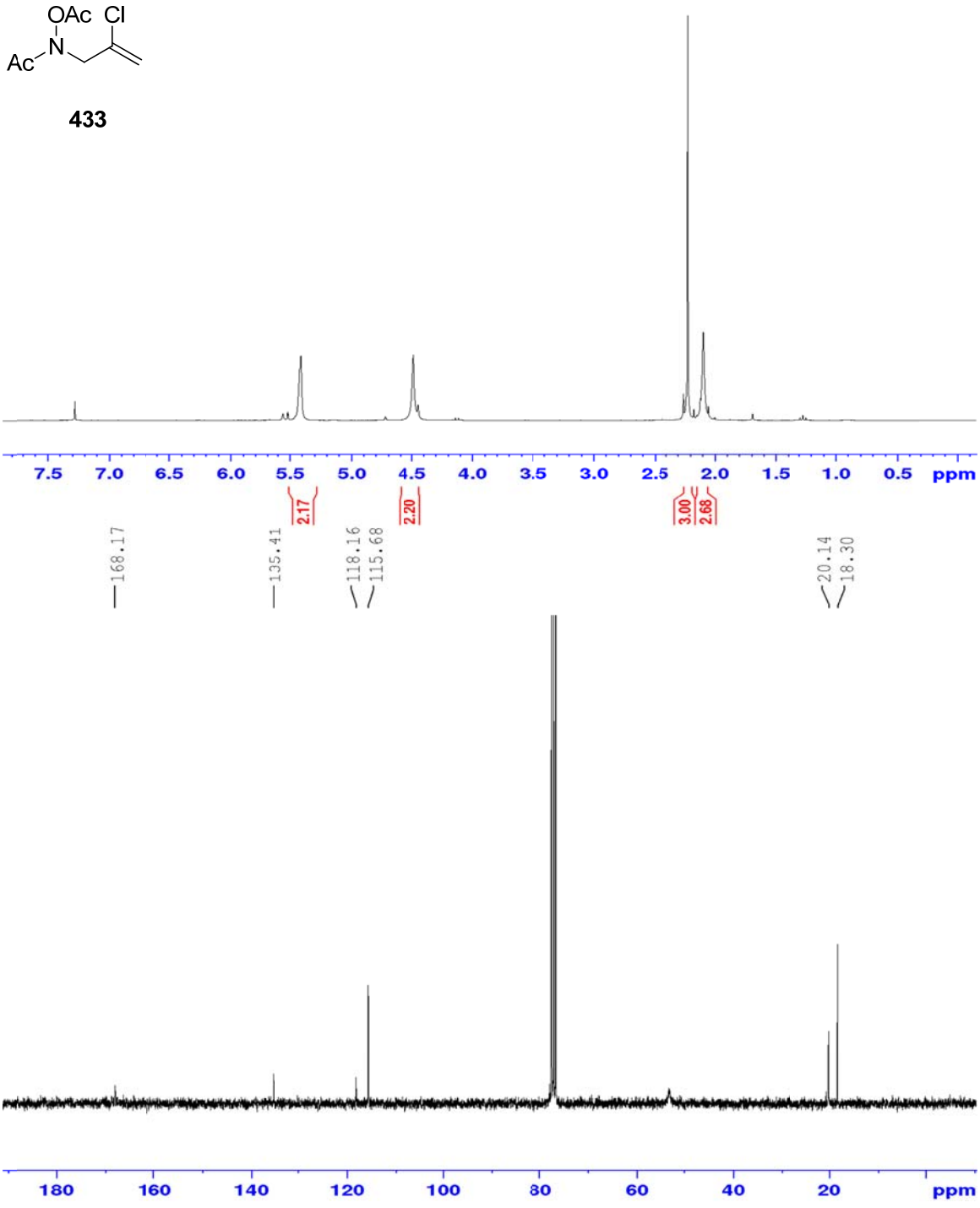
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
1.03
1.02
2.00

432


OAc
Cl
N
Ac
433
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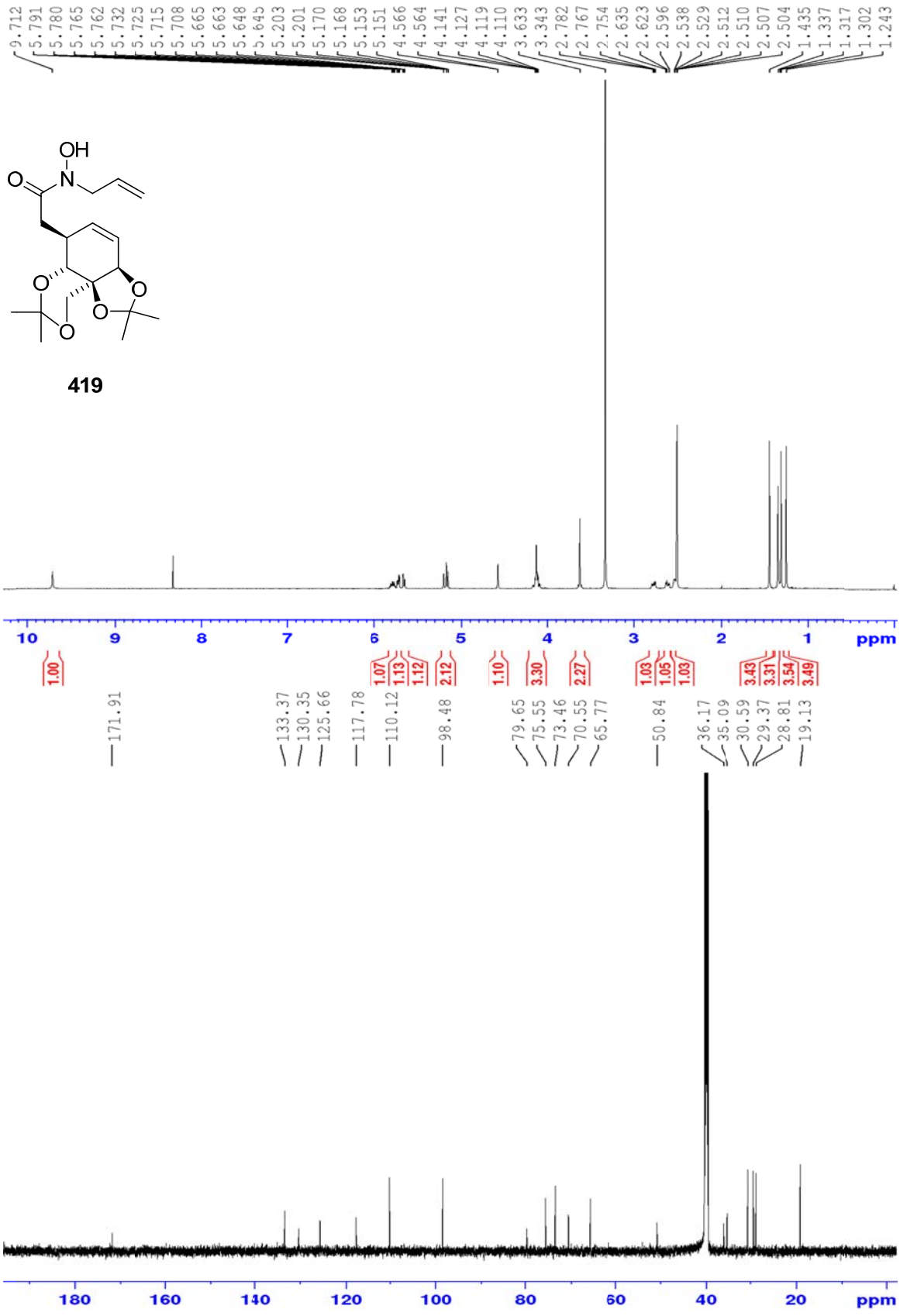
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
2.17
2.20
3.00
2.68
168.17
135.41
118.16
115.68
20.14
18.30
180
160
140
120
100
80
60
40
20
ppm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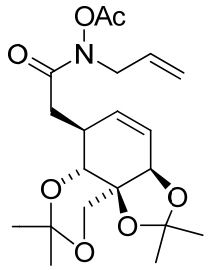
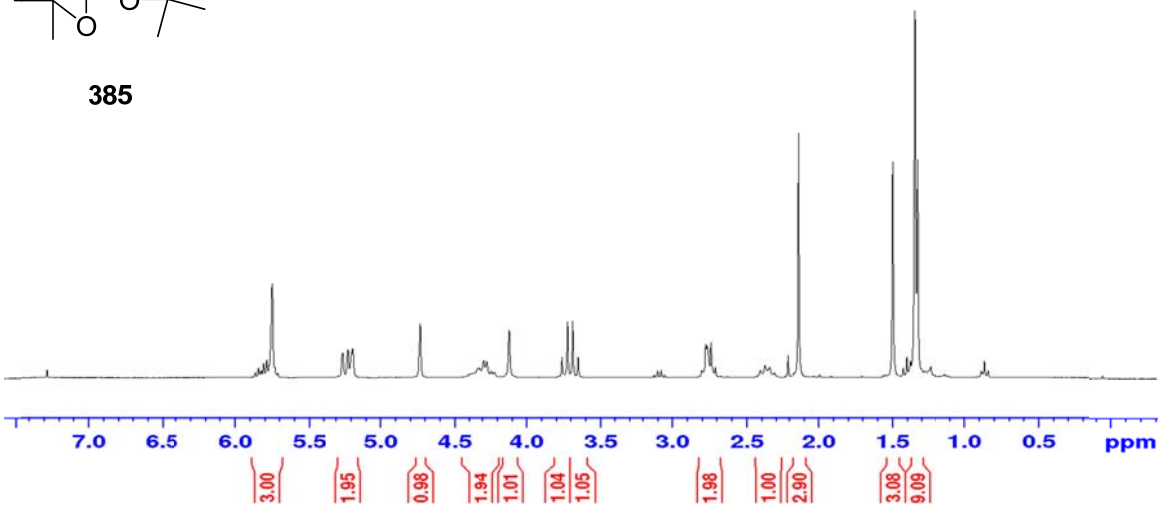
5.765
5.762
5.732
5.725
5.715
5.708
5.665
5.663
5.648
5.645
5.203
5.201
5.170
5.168
5.153
5.151
4.566
4.564
4.141
4.127
4.119
4.110
3.633
3.343
2.782
2.767
2.754
2.635
2.623
2.596
2.538
2.529
2.512
2.510
2.507
2.504
1.435
1.337
1.317
1.302
1.243
OH
N
O
O
O
O
O
10
9
8
7
6
5
4
3
2
1
ppm
1.00
1.07
1.13
1.12
2.12
1.10
3.30
2.27
1.03
1.05
1.03
3.43
3.31
3.54
3.49
171.91
133.37
130.35
125.66
117.78
110.12
98.48
79.65
75.55
73.46
70.55
65.77
50.84
36.17
35.09
30.59
29.37
28.81
19.13
180
160
140
120
100
80
60
40
20
ppm

419


5.843
5.809
5.787
5.773
5.751
5.733
5.254
5.222
5.196
5.188
4.733
4.327
4.292
4.271
4.239
4.218
4.118
3.760
3.723
3.687
3.650
2.802
2.774
2.765
2.739
2.710
2.407
2.376
2.344
2.312
2.135
1.496
1.346
1.328


OAc
O
N
O
O
O
O

385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6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
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1.94
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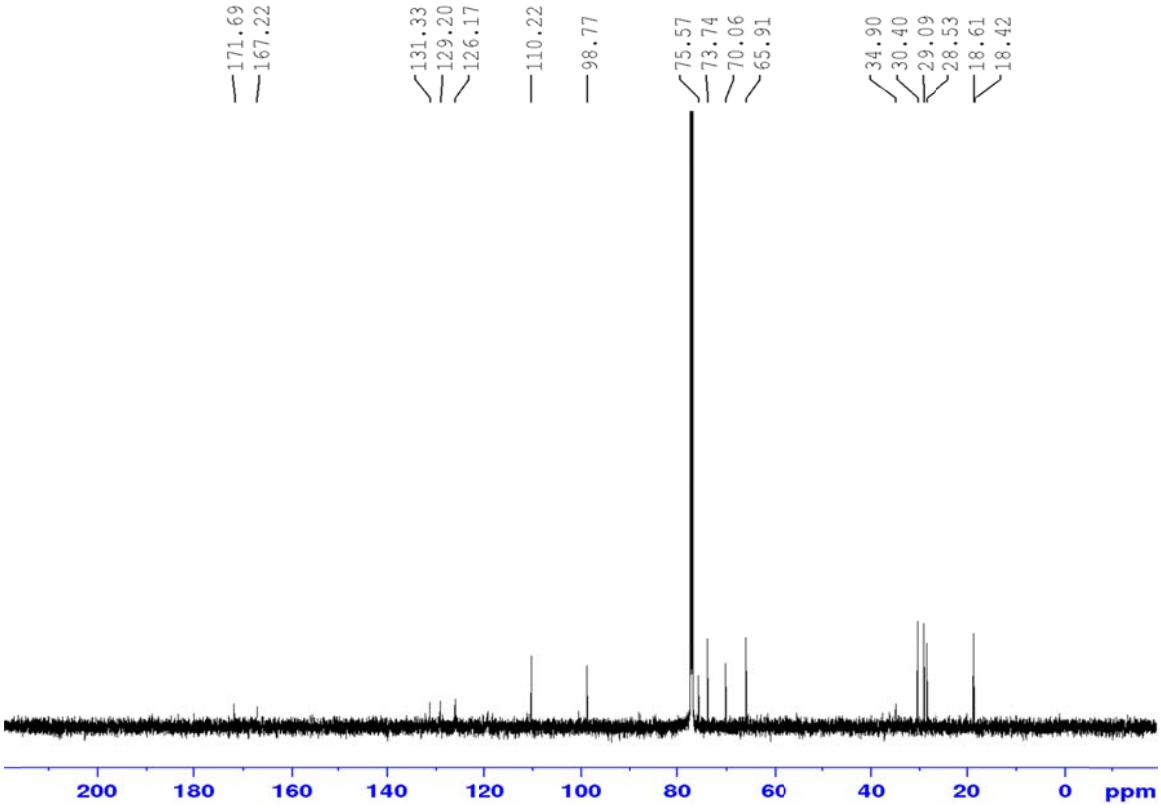
1.04
1.05
1.98
1.00
2.90
3.08
9.09


171.69
167.22
131.33
129.20
126.17
110.22
98.77
75.57
73.74
70.06
65.91
34.90
30.40
29.09
28.53
18.61
18.42
200
180
160
140
120
100
80
60
40
20
0
ppm


9.884
5.762
5.748
5.728
5.713
5.681
5.673
5.645
5.638
5.484
5.463
5.414
5.410
4.566
4.407
4.354
4.317
4.263
4.219
4.128
3.632
3.333
2.865
2.839
2.809
2.784
2.696
2.672
2.617
2.578
2.557
2.541
2.509
2.498
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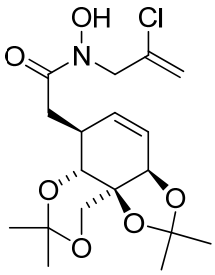
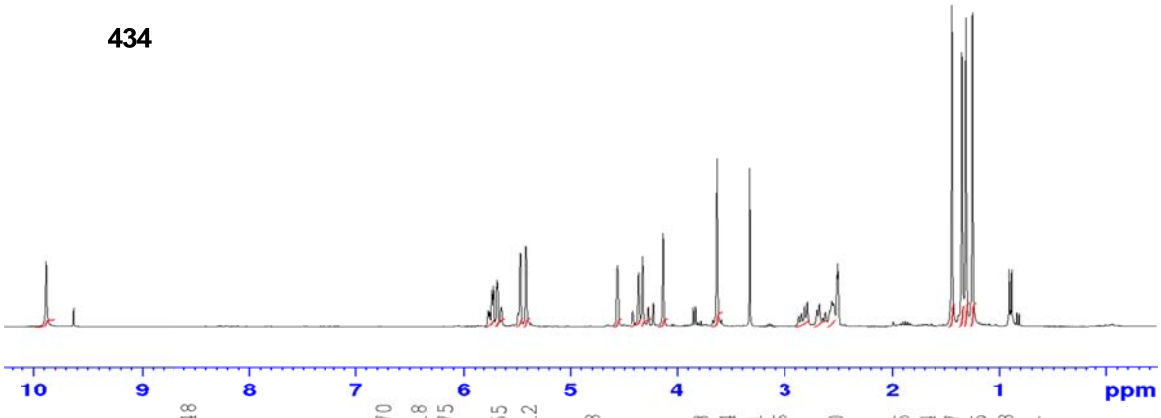
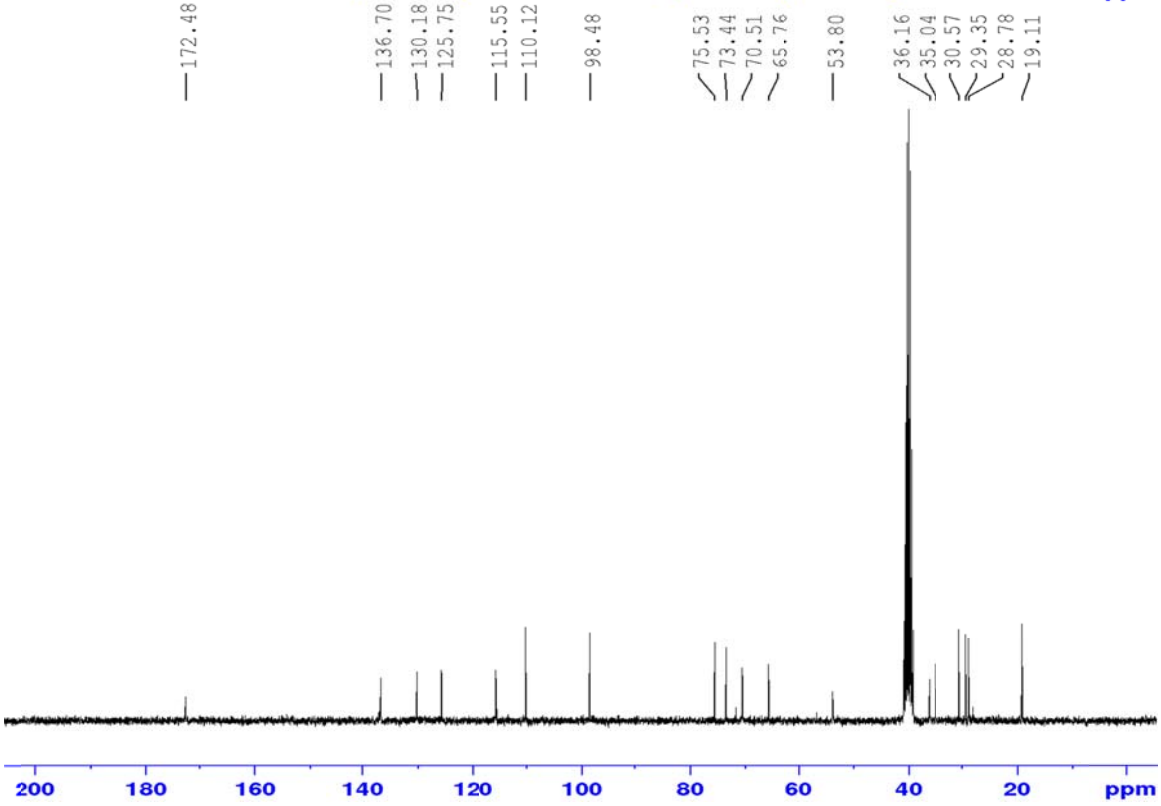
1.339


OH
Cl
O
N
O
O
O
O

434


10
9
8
7
6
5
4
3
2
1
ppm


172.48
136.70
130.18
125.75
115.55
110.12
98.48
75.53
73.44
70.51
65.76
53.80
36.16
35.04
30.57
29.35
28.78
19.11
200
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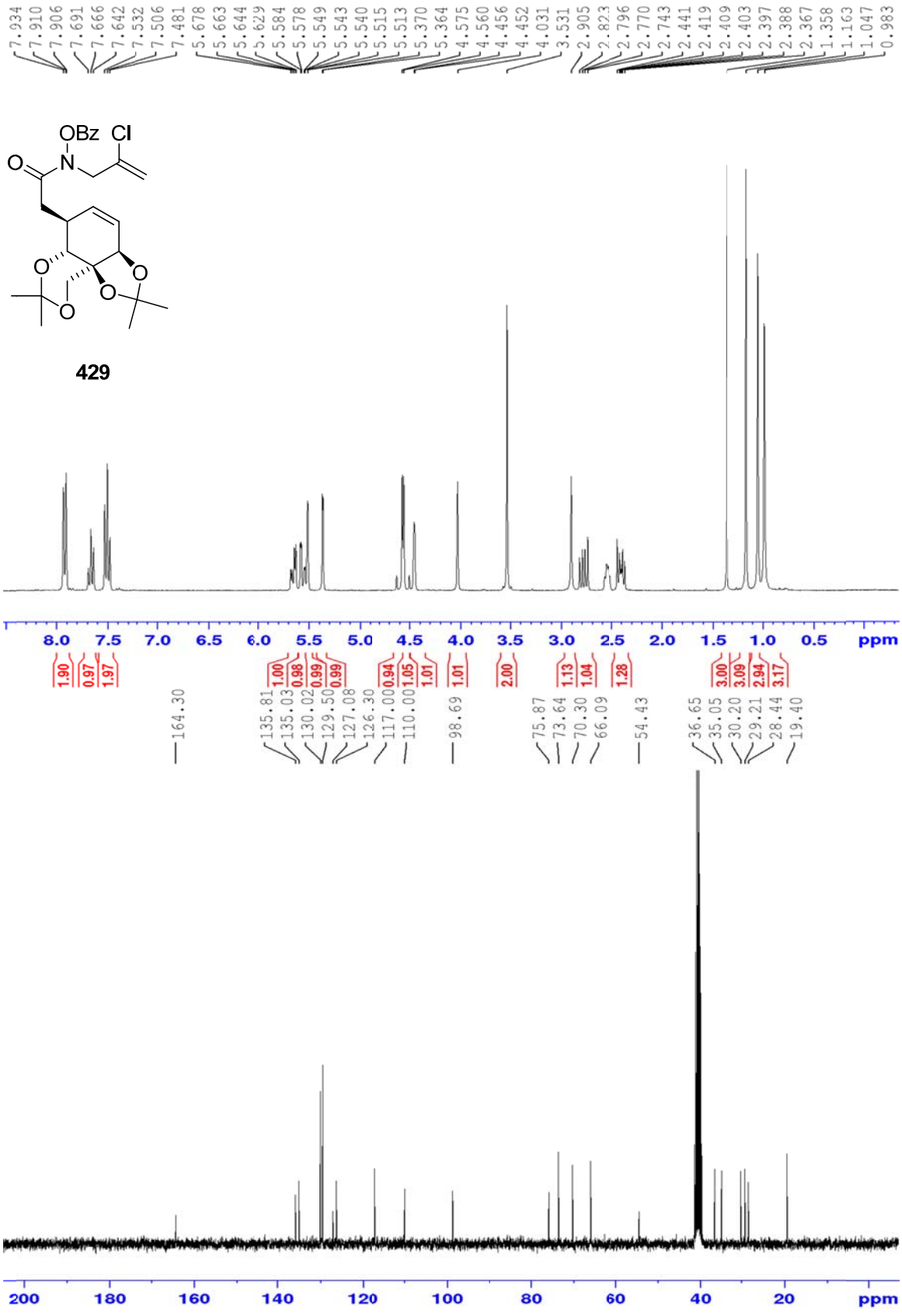
160
140
120
100
80
60
40
20
ppm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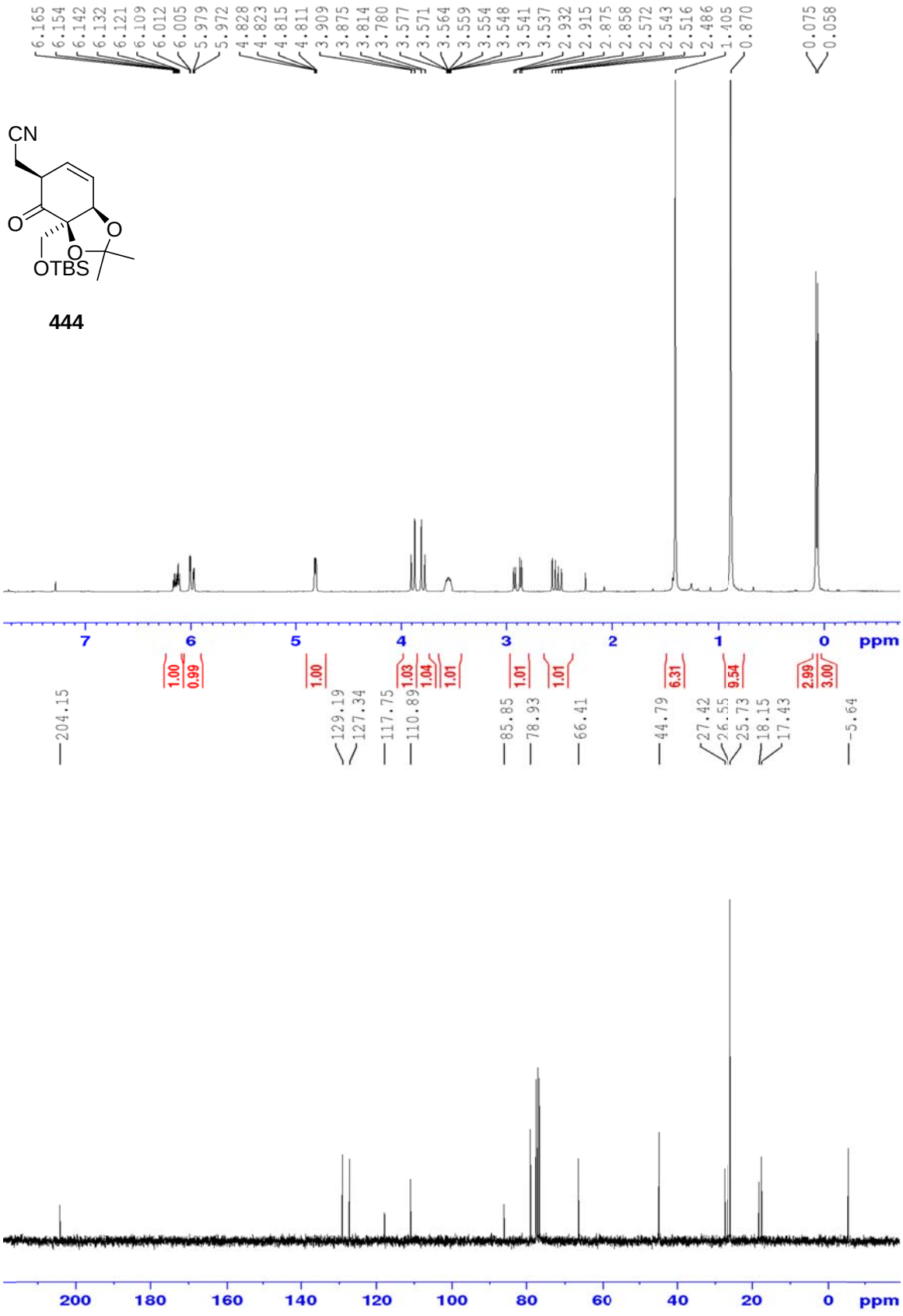
7.666
7.642
7.532
7.506
7.481
5.678
5.663
5.644
5.629
5.584
5.578
5.549
5.543
5.540
5.515
5.513
5.370
5.364
4.575
4.560
4.456
4.452
4.031
3.531
2.905
2.823
2.796
2.770
2.743
2.441
2.419
2.409
2.403
2.397
2.388
2.367
1.358
1.163
1.047
0.983
OBz
Cl
O
N
O
O
O
O
429
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
1.90
0.97
1.97
1.00
0.98
0.99
0.99
0.94
1.05
1.01
1.01
2.00
1.13
1.04
1.28
3.00
3.09
2.94
3.17
164.30
135.81
135.03
130.02
129.50
127.08
126.30
117.00
110.00
98.69
75.87
73.64
70.30
66.09
54.43
36.65
35.05
30.20
29.21
28.44
19.40
200
180
160
140
120
100
80
60
40
20
ppm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4.823
4.815
4.811
3.909
3.875
3.814
3.780
3.577
3.571
3.564
3.559
3.554
3.548
3.541
3.537
2.932
2.915
2.875
2.858
2.572
2.543
2.516
2.486
1.405
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0.075
0.058
CN
O
O
O
OTBS
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6
5
4
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ppm
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1.00
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1.04
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1.01
1.01
1.01
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3.00
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ppm

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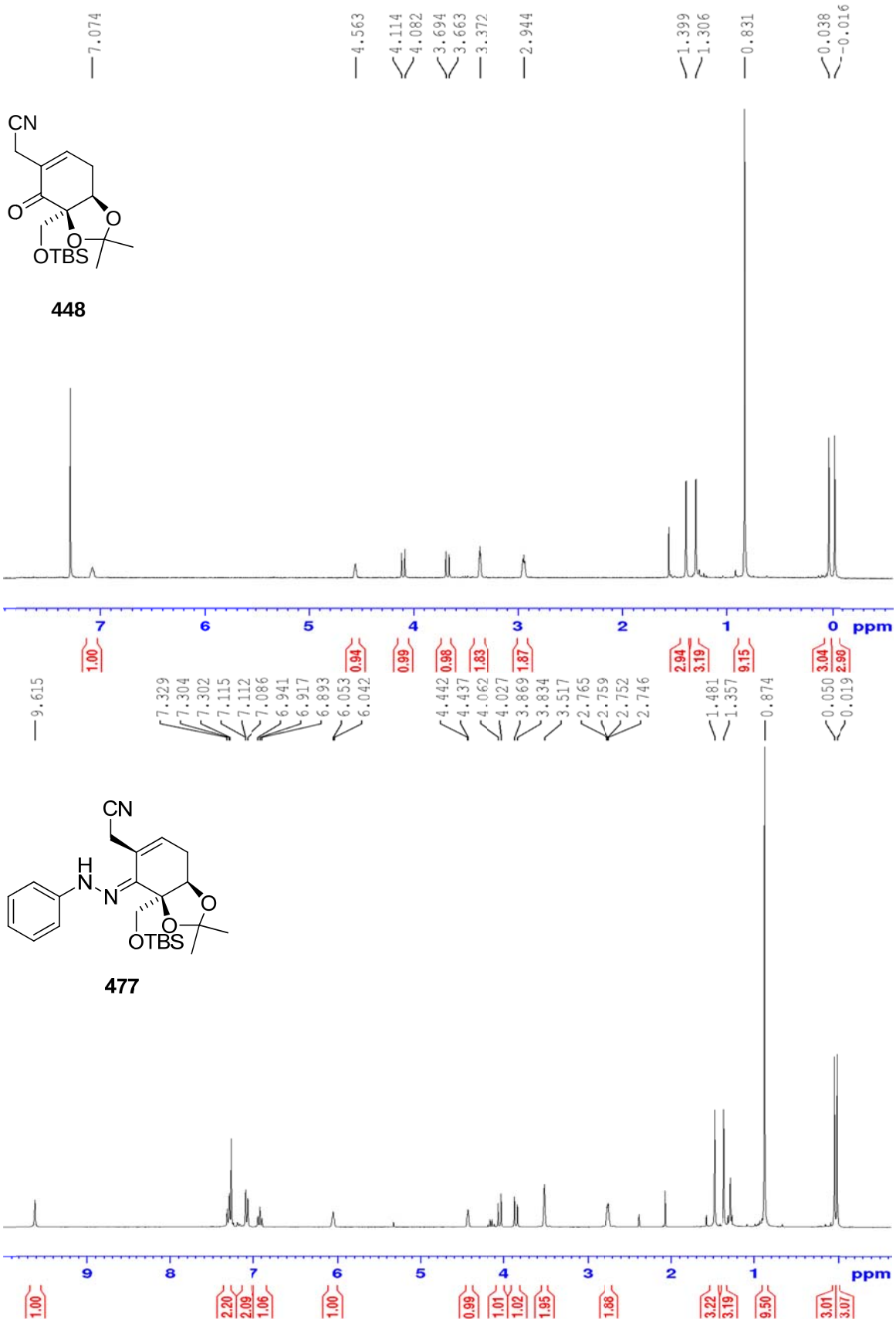
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CN
O
O
O
OTBS
448
ppm
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7.304
7.302
7.115
7.112
7.086
6.941
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6.893
6.053
6.042
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4.062
4.027
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3.834
3.517
2.765
2.759
2.752
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H
N
N
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O
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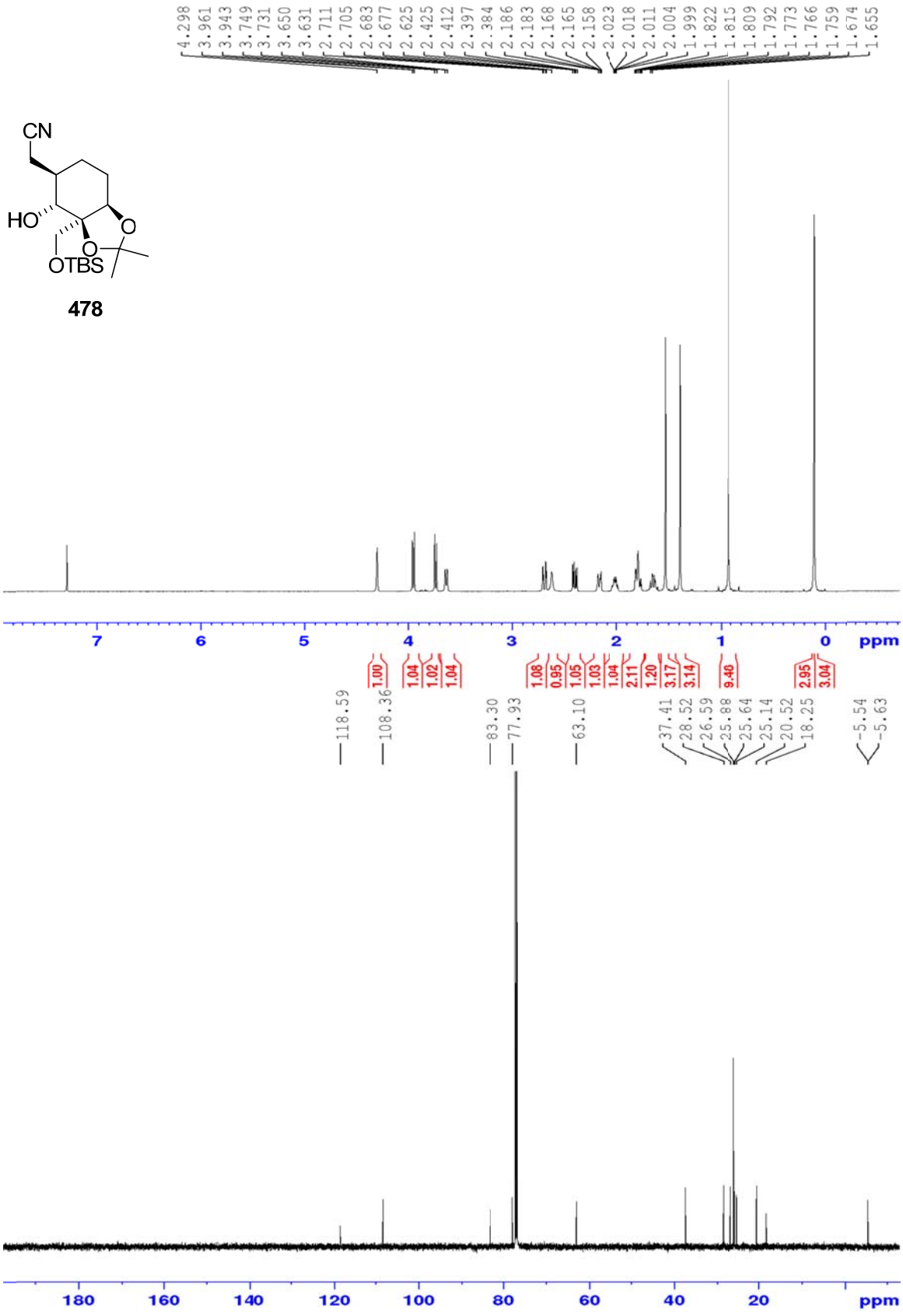
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2.425
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2.397
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2.186
2.183
2.168
2.165
2.158
2.023
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2.011
2.004
1.999
1.822
1.815
1.809
1.792
1.773
1.766
1.759
1.674
1.655
CN
HO
O
O
OTBS
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6
5
4
3
2
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ppm
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1.04
1.08
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1.05
1.03
1.04
2.11
1.20
3.17
3.14
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2.95
3.04
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108.36
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77.93
63.10
37.41
28.52
26.59
25.88
25.64
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120
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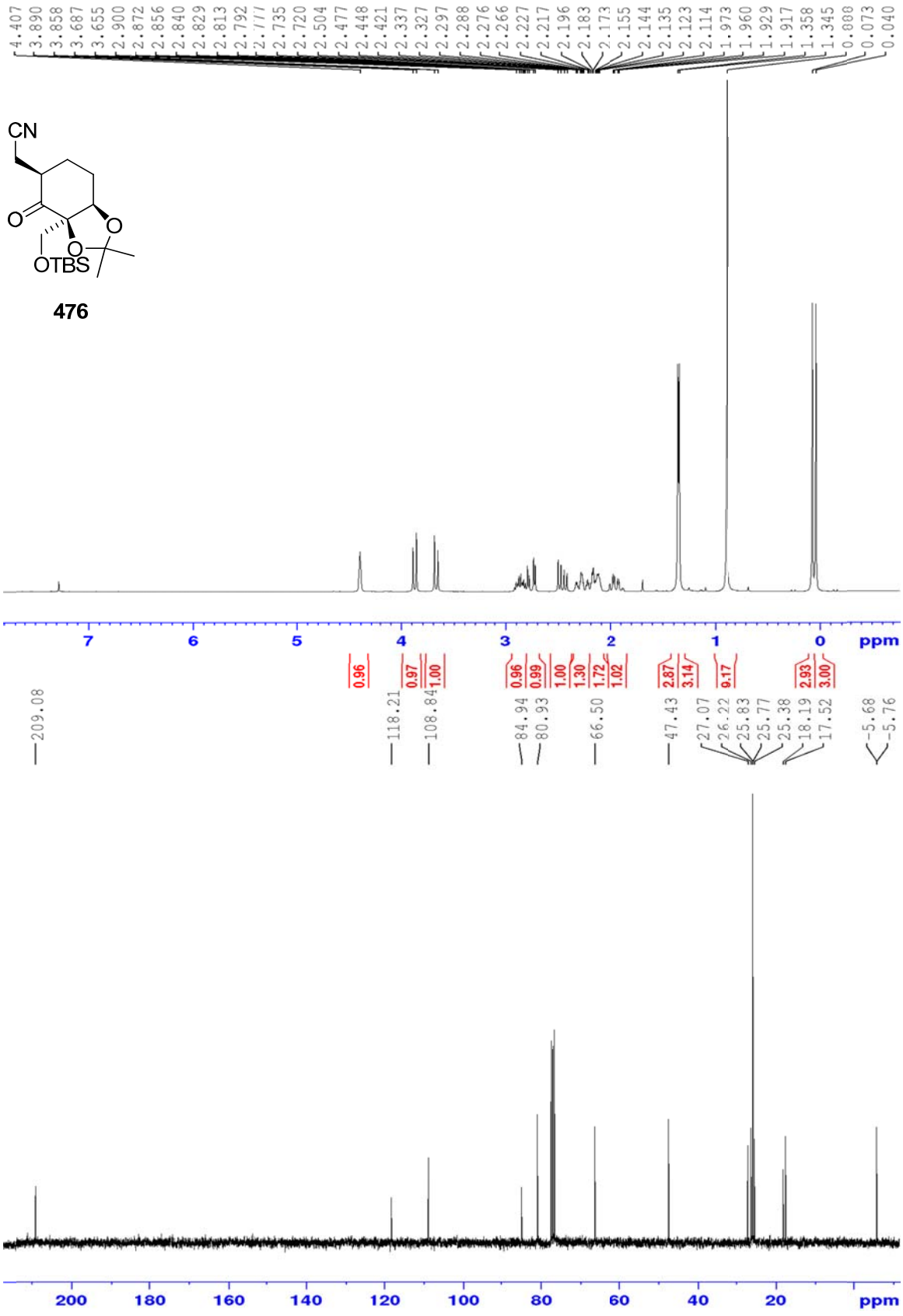
60
40
20
ppm

478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2.276
2.266
2.227
2.217
2.196
2.183
2.173
2.155
2.144
2.135
2.123
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1.917
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O
O
O
OTBS
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7
6
5
4
3
2
1
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ppm
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0.97
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2.93
3.00
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26.22
25.83
25.77
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20
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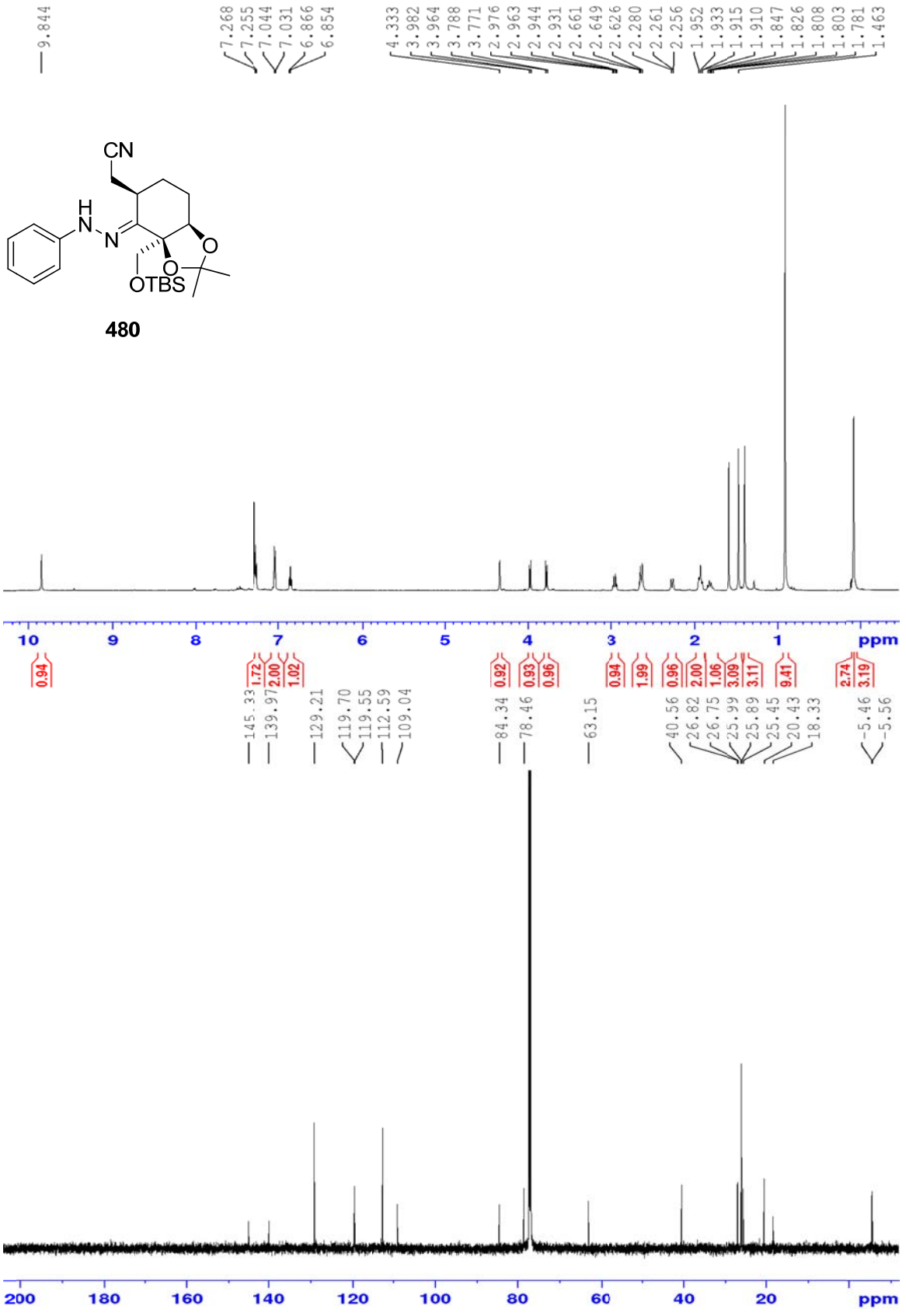
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2.976
2.963
2.944
2.931
2.661
2.649
2.626
2.280
2.261
2.256
1.952
1.933
1.915
1.910
1.847
1.826
1.808
1.803
1.781
1.463
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H
N
N
O
O
OTBS
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8
7
6
5
4
3
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ppm
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1.02
0.92
0.93
0.96
0.94
1.99
0.96
2.00
1.06
3.09
3.11
9.41
2.74
3.19
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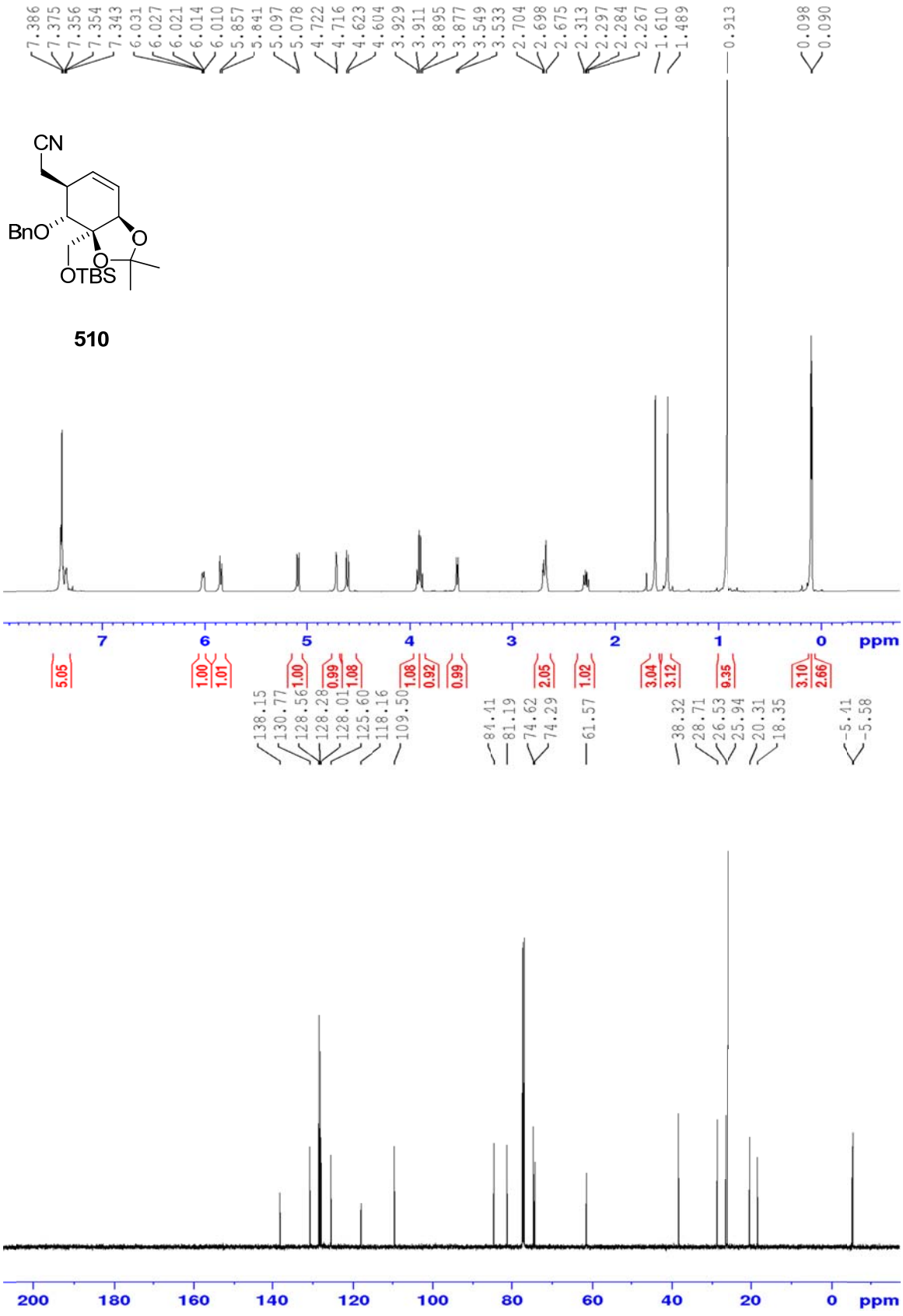
129.21
119.70
119.55
112.59
109.04
84.34
78.46
63.15
40.56
26.82
26.75
25.99
25.89
25.45
20.43
18.33
-5.46
-5.56
200
180
160
140
120
100
80
60
40
20
ppm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5.078
4.722
4.716
4.623
4.604
3.929
3.911
3.895
3.877
3.549
3.533
2.704
2.698
2.675
2.313
2.297
2.284
2.267
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1.489
0.913
0.098
0.090
CN
BnO
O
O
OTBS
510
ppm
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1.01
1.00
0.99
1.08
1.08
0.92
0.99
2.05
1.02
3.04
3.12
9.35
3.10
2.66
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130.77
128.56
128.28
128.01
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81.19
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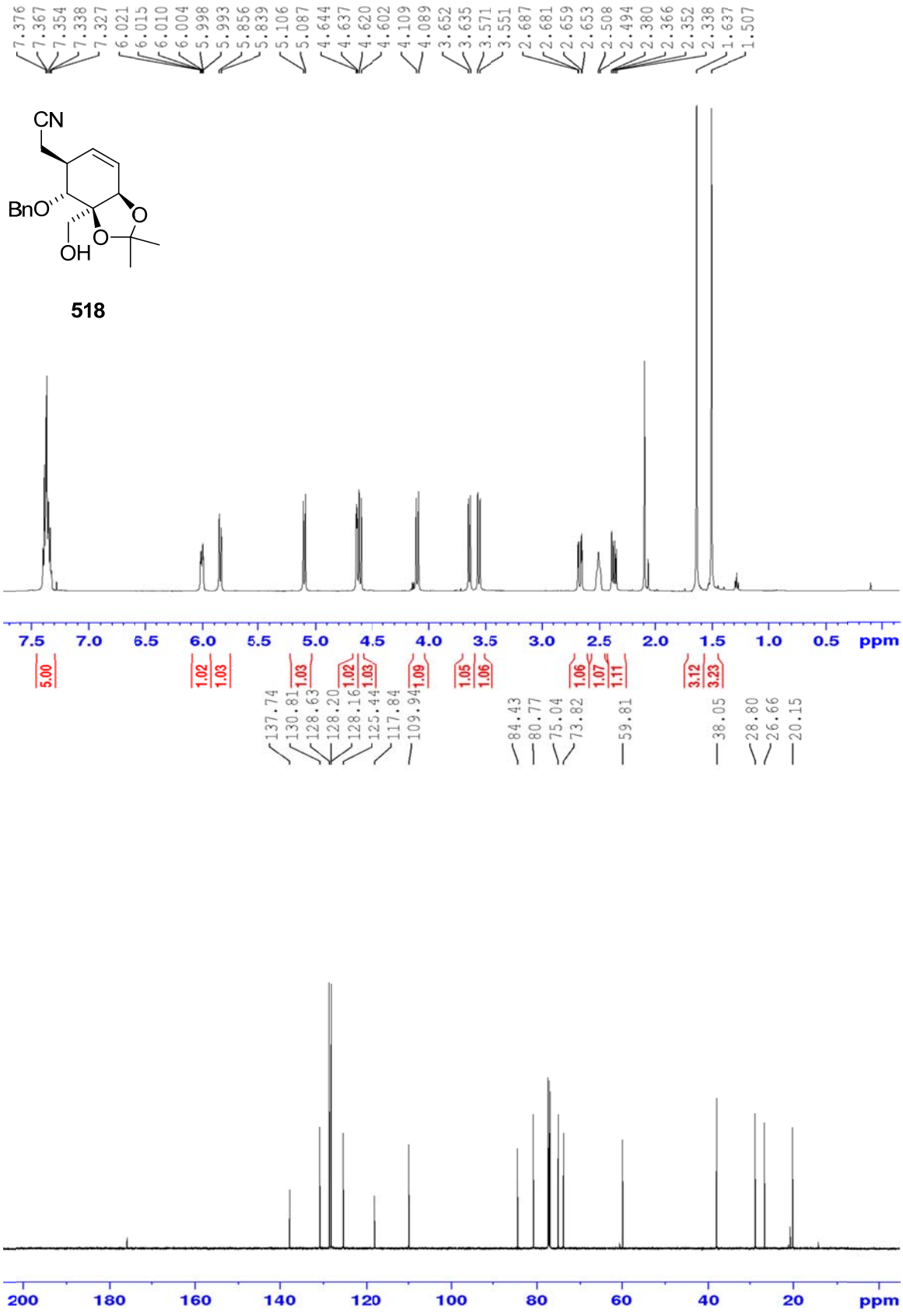
ppm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5.839
5.106
5.087
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4.637
4.620
4.602
4.109
4.089
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3.635
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3.551
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2.659
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2.508
2.494
2.380
2.366
2.352
2.338
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1.507
CN
BnO
O
O
OH
518
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6.0
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5.0
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3.0
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1.5
1.0
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ppm
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1.03
1.03
1.02
1.03
1.09
1.05
1.06
1.06
1.07
1.11
3.12
3.23
137.74
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128.20
128.16
125.44
117.84
109.94
84.43
80.77
75.04
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26.66
20.15
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160
140
120
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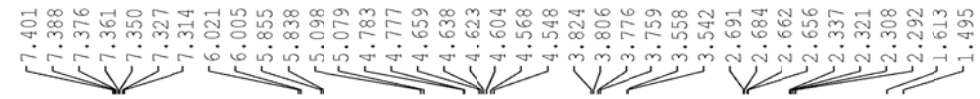
60
40
20
ppm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4.548
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3.806
3.776
3.759
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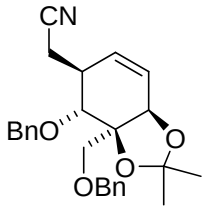
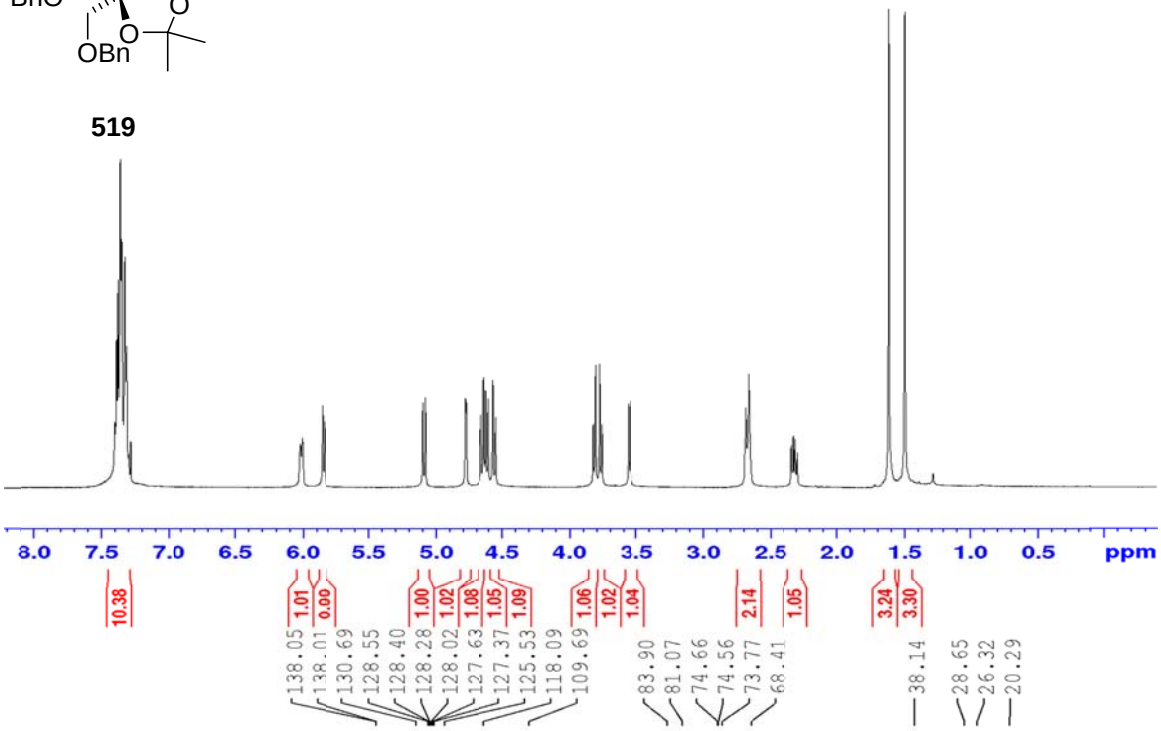
1.495


CN
BnO
O
O
OBn

519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2.0
1.5
1.0
0.5
ppm
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0.00
1.00
1.02
1.08
1.05
1.09
1.06
1.02
1.04
2.14
1.05
3.24
3.30
138.05
138.01
130.69
128.55
128.40
128.28
128.02
127.63
127.37
125.53
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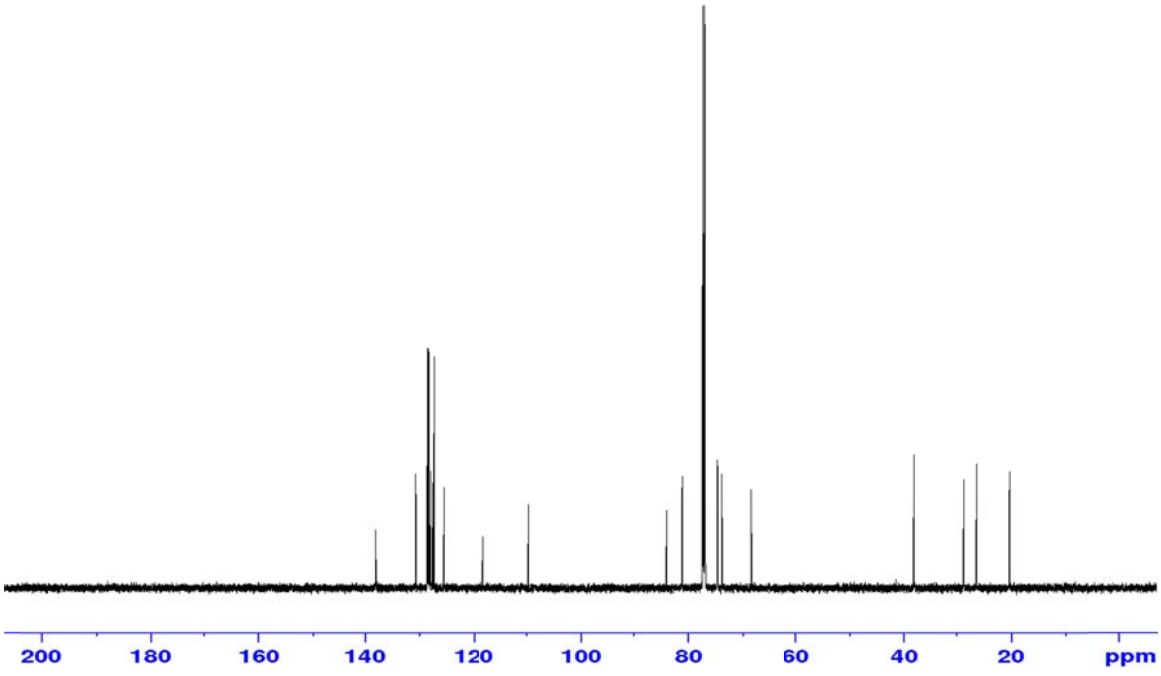
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60
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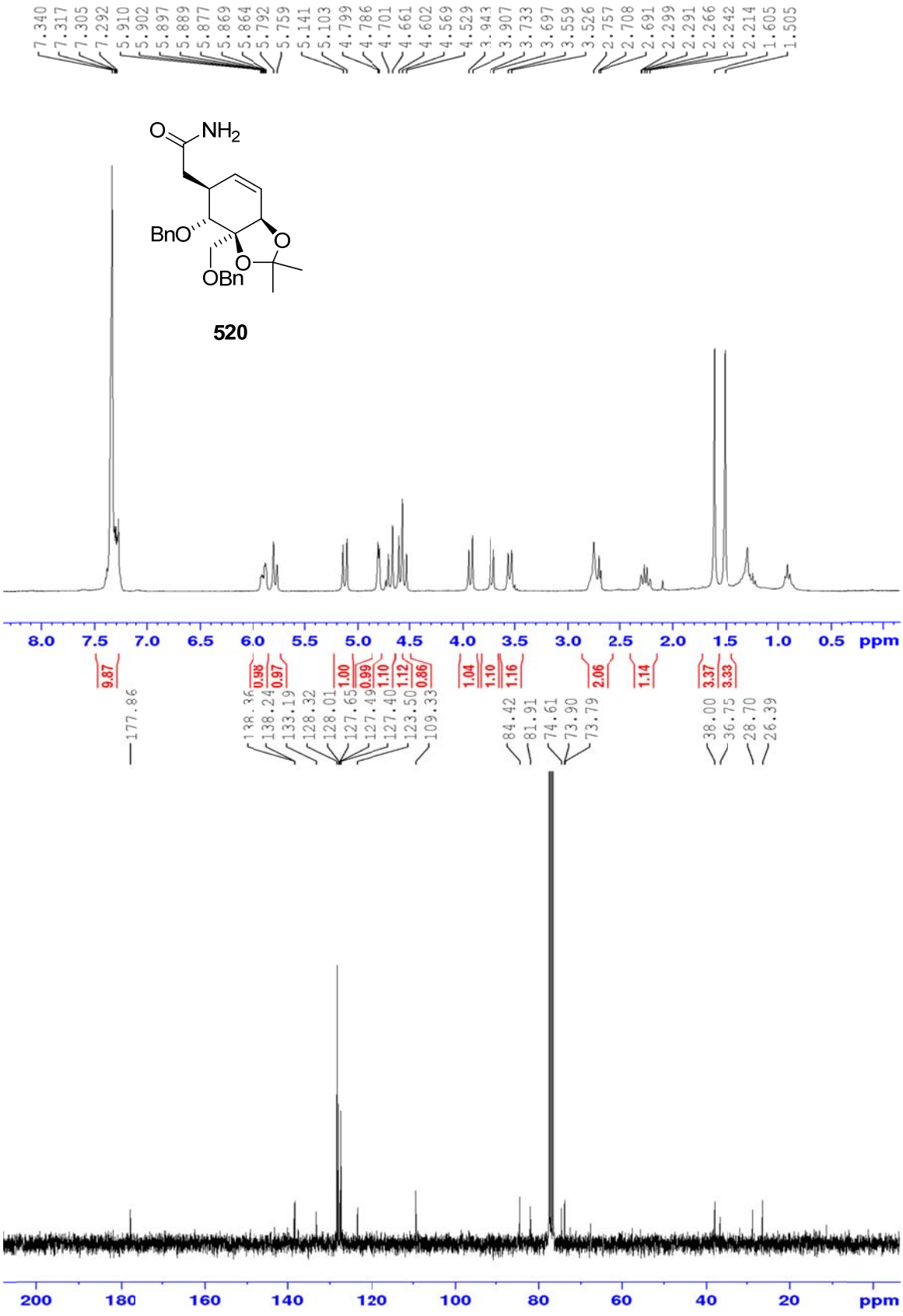
20
ppm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2.266
2.242
2.214
1.605
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O
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BnO
O
O
OBn
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3.33
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127.40
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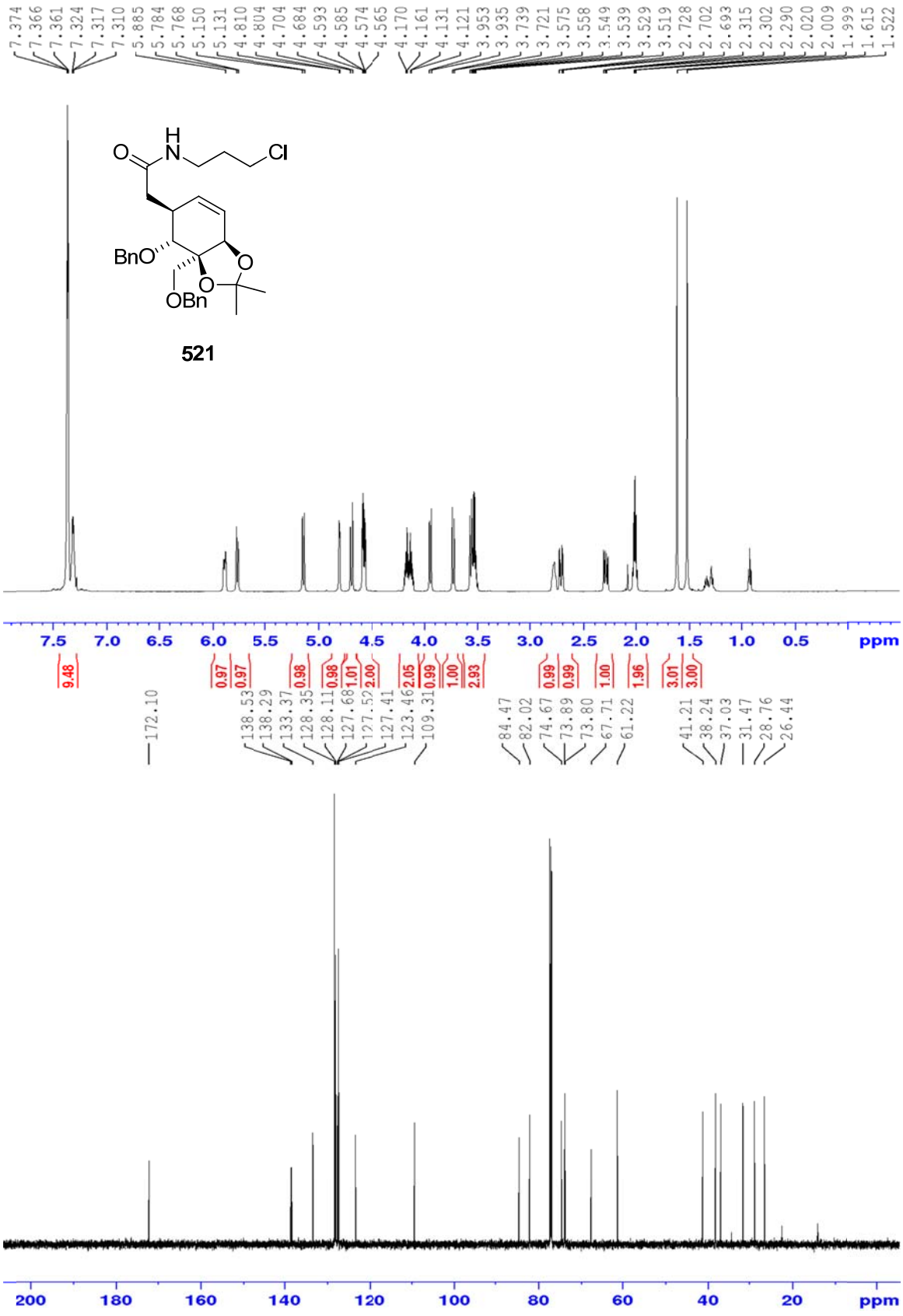
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4.161
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2.315
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2.290
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2.009
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1.522
H
N
O
Cl
BnO
O
O
OBn
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127.41
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73.89
73.80
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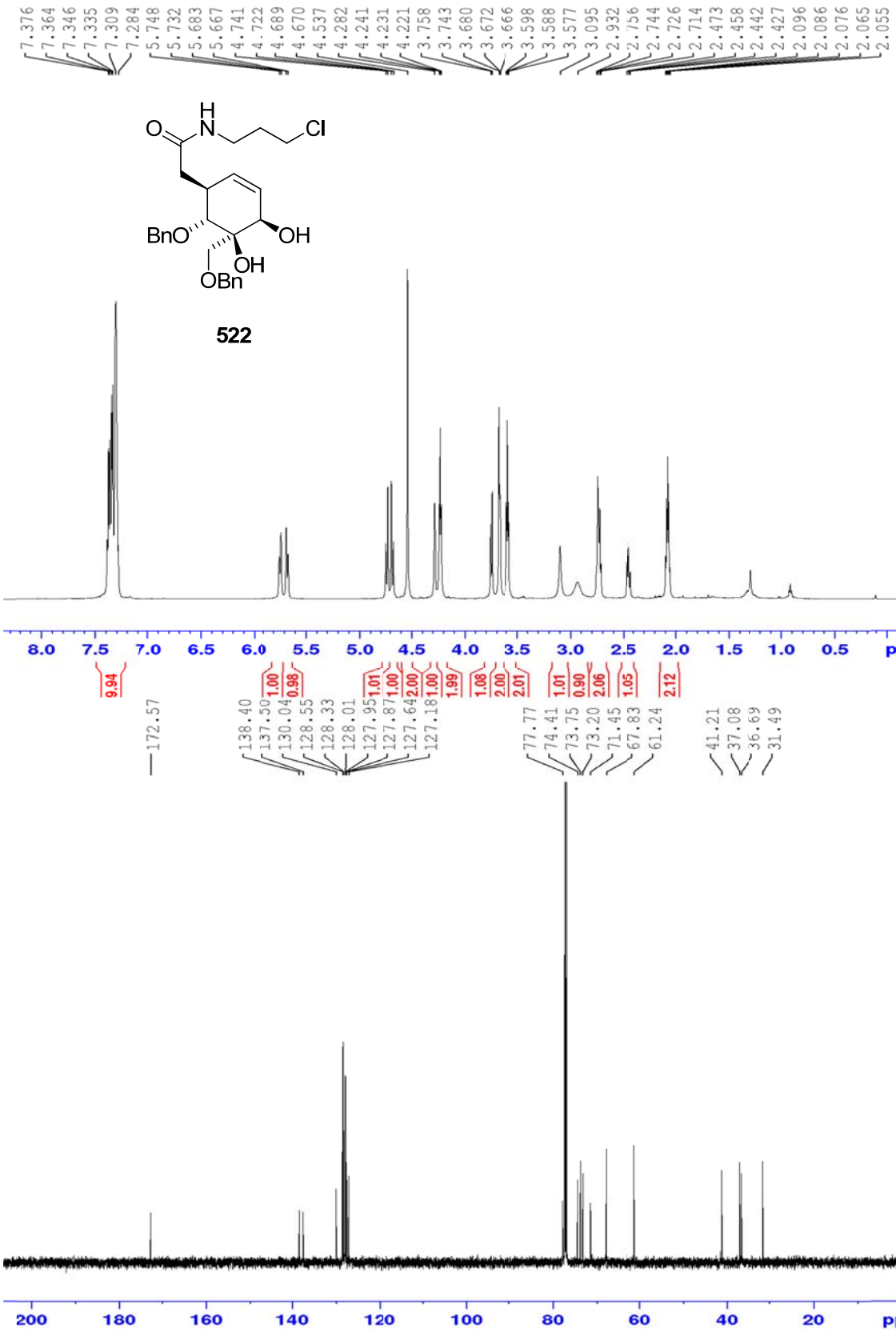
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N
O
Cl
BnO
OH
OH
OBn
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127.18
77.77
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73.75
73.20
71.45
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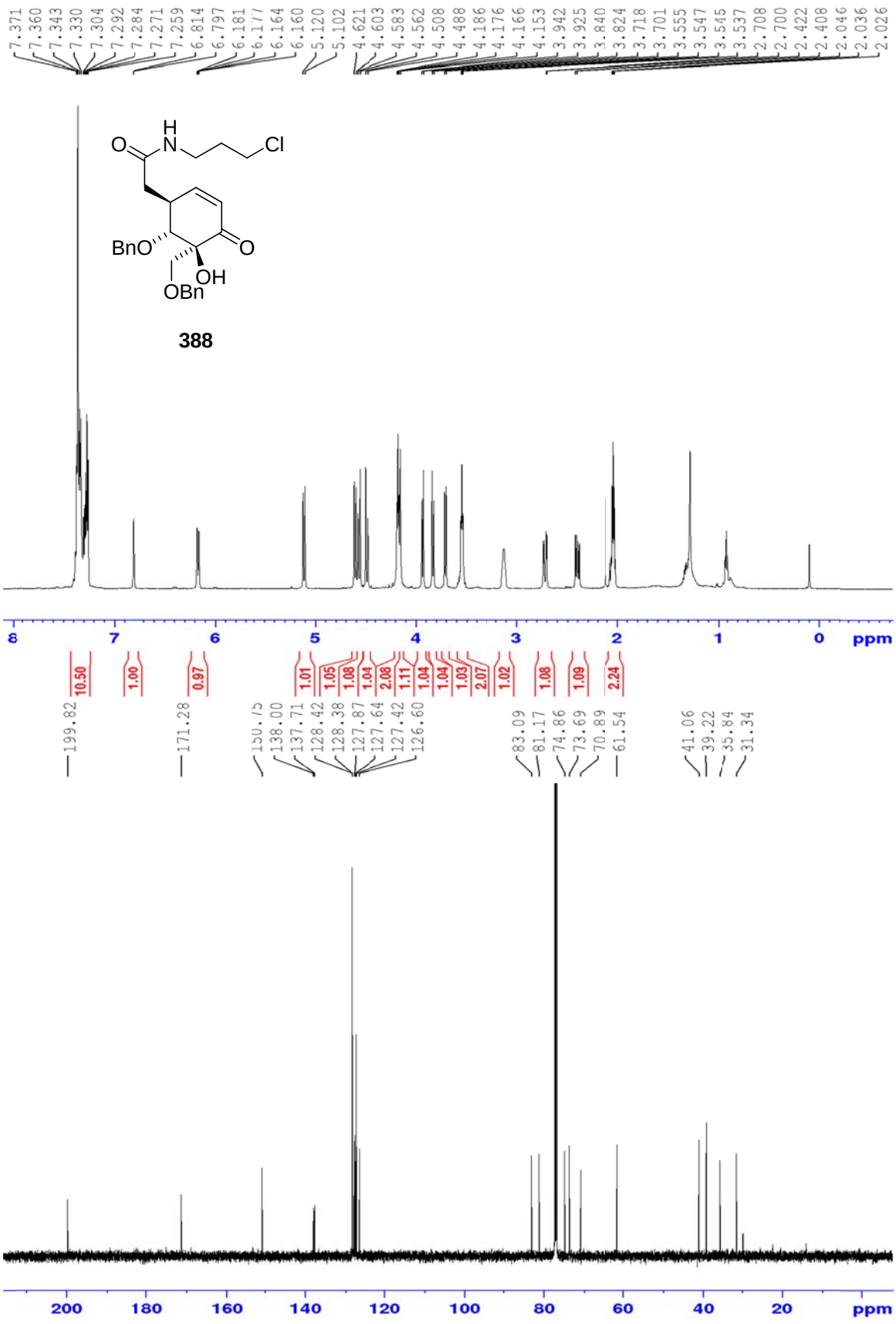
20
ppm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H
N
O
Cl
BnO
O
OH
OBn
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1.04
2.08
1.11
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1.04
1.03
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1.02
1.08
1.09
2.24
199.82
171.28
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128.38
127.87
127.64
127.42
126.60
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81.17
74.86
73.69
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31.34
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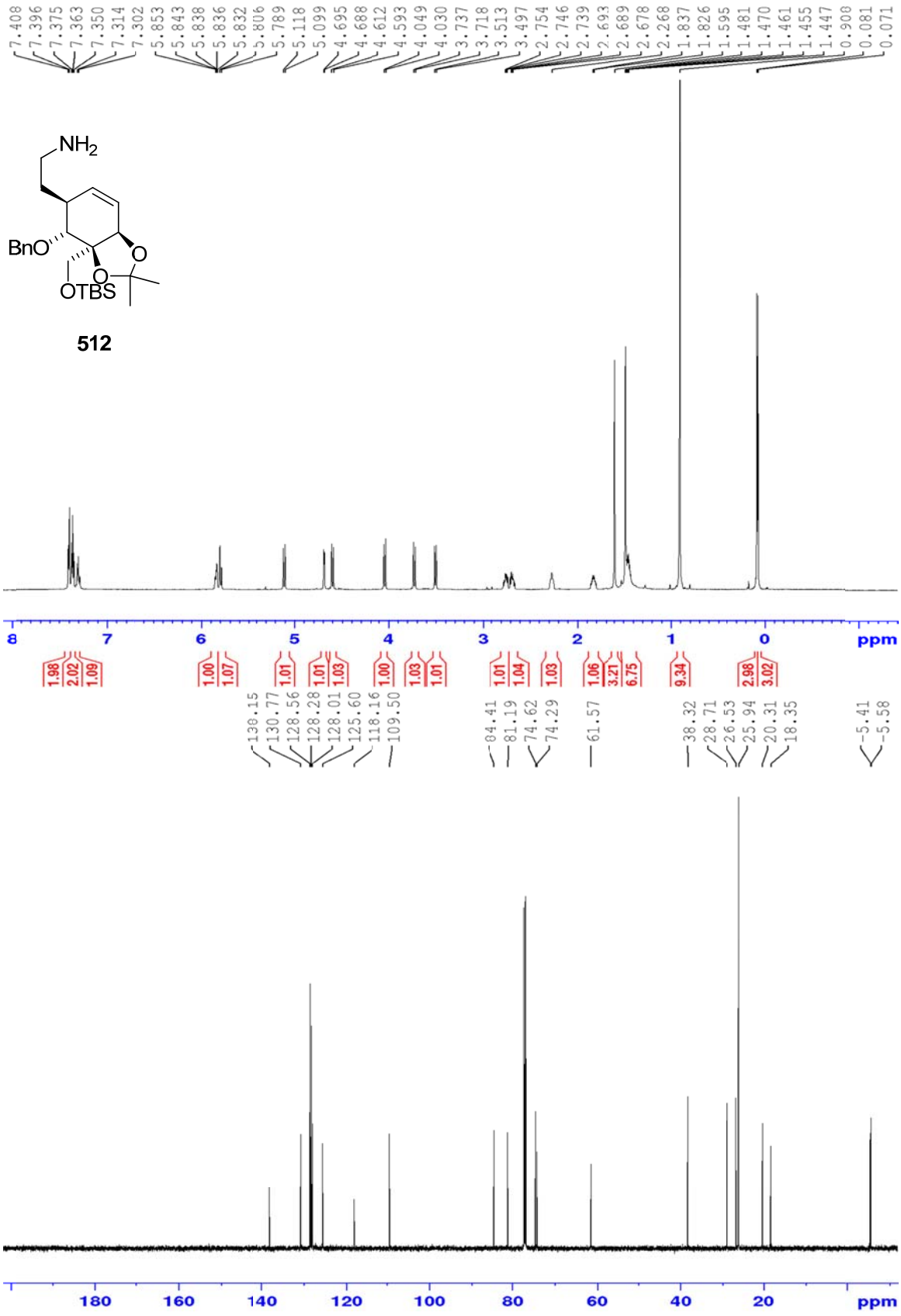
60
40
20
ppm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5.099
4.695
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3.513
3.497
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2.746
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0.071
NH2
BnO
O
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OTBS
512
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ppm
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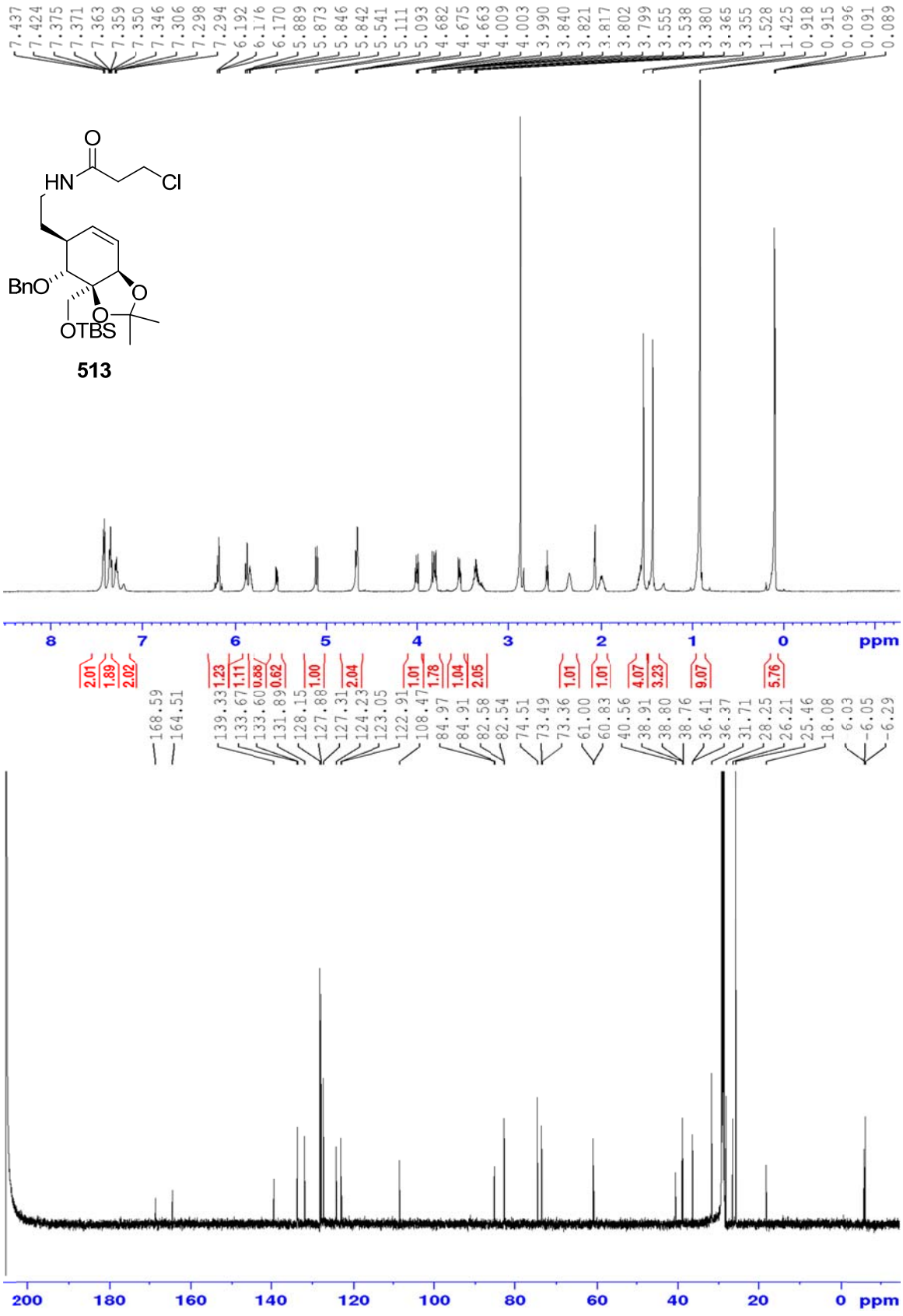
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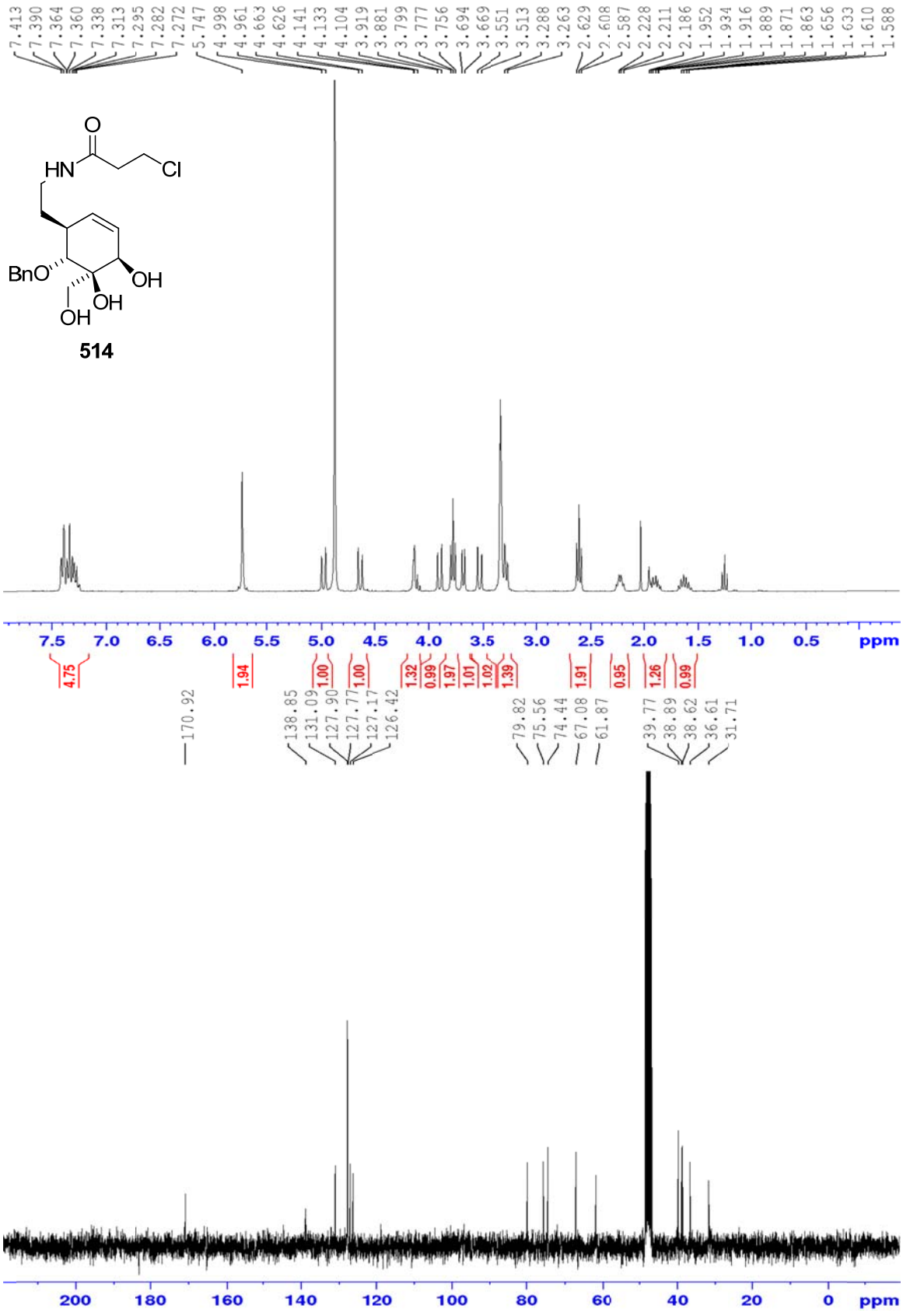
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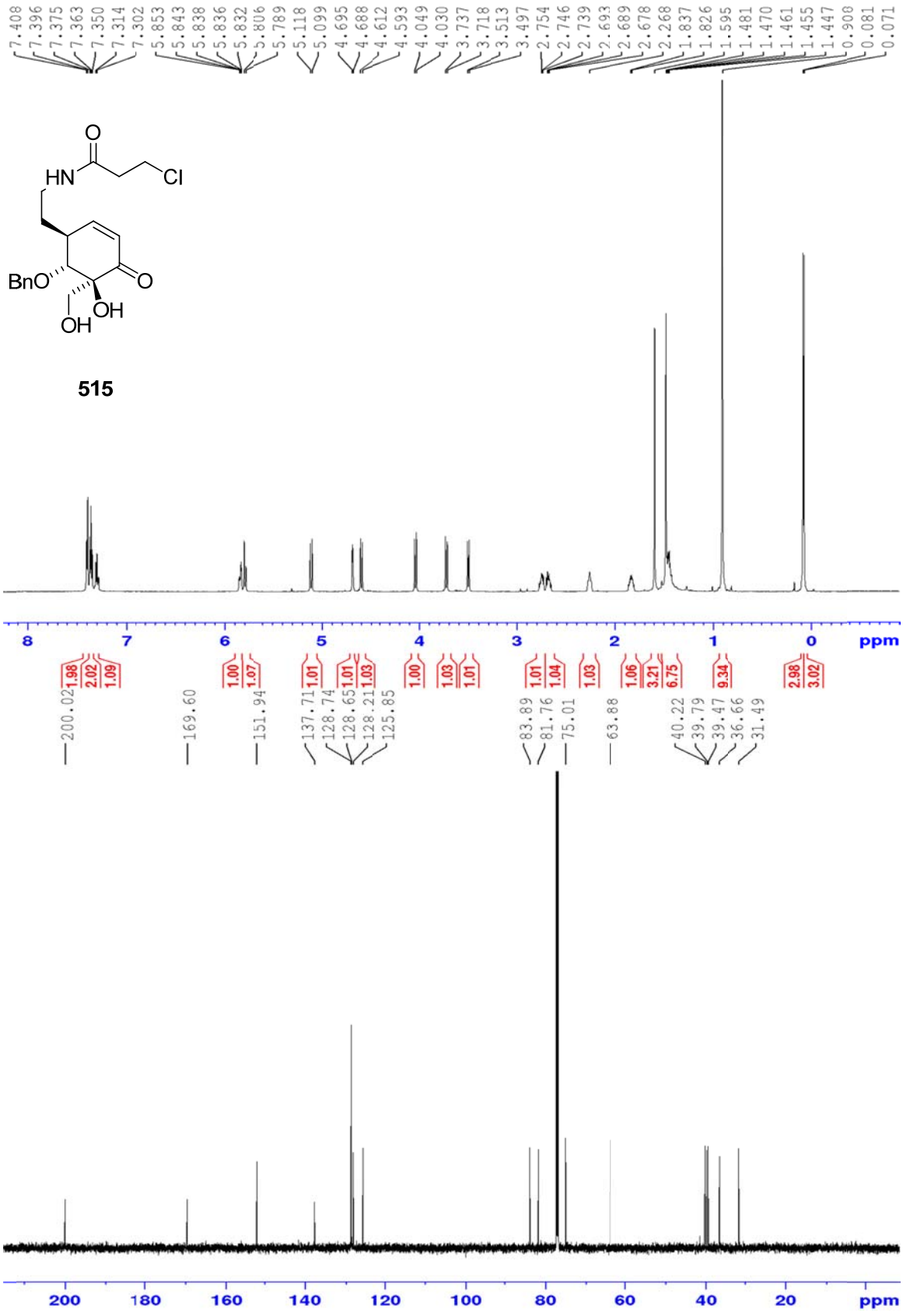
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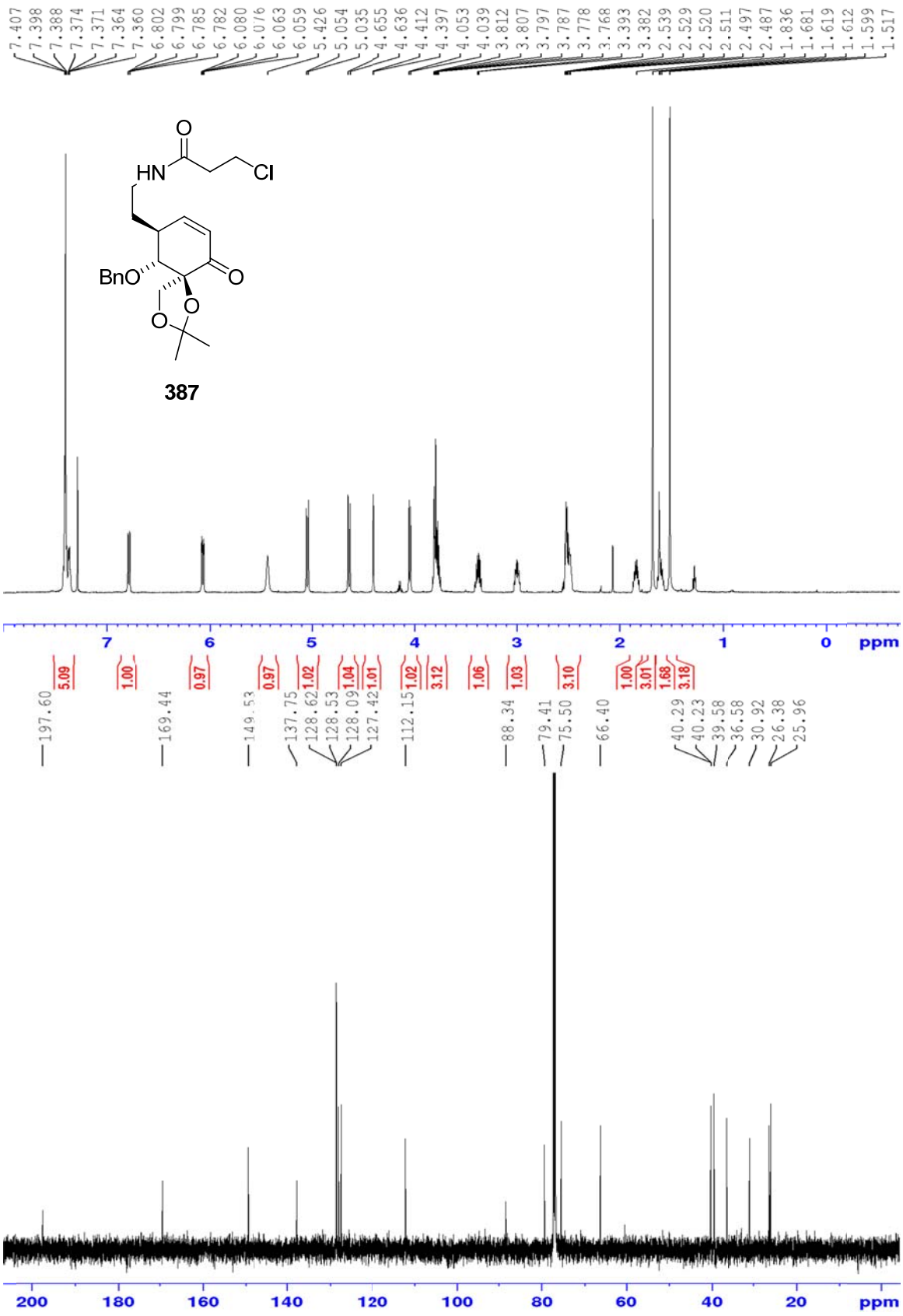
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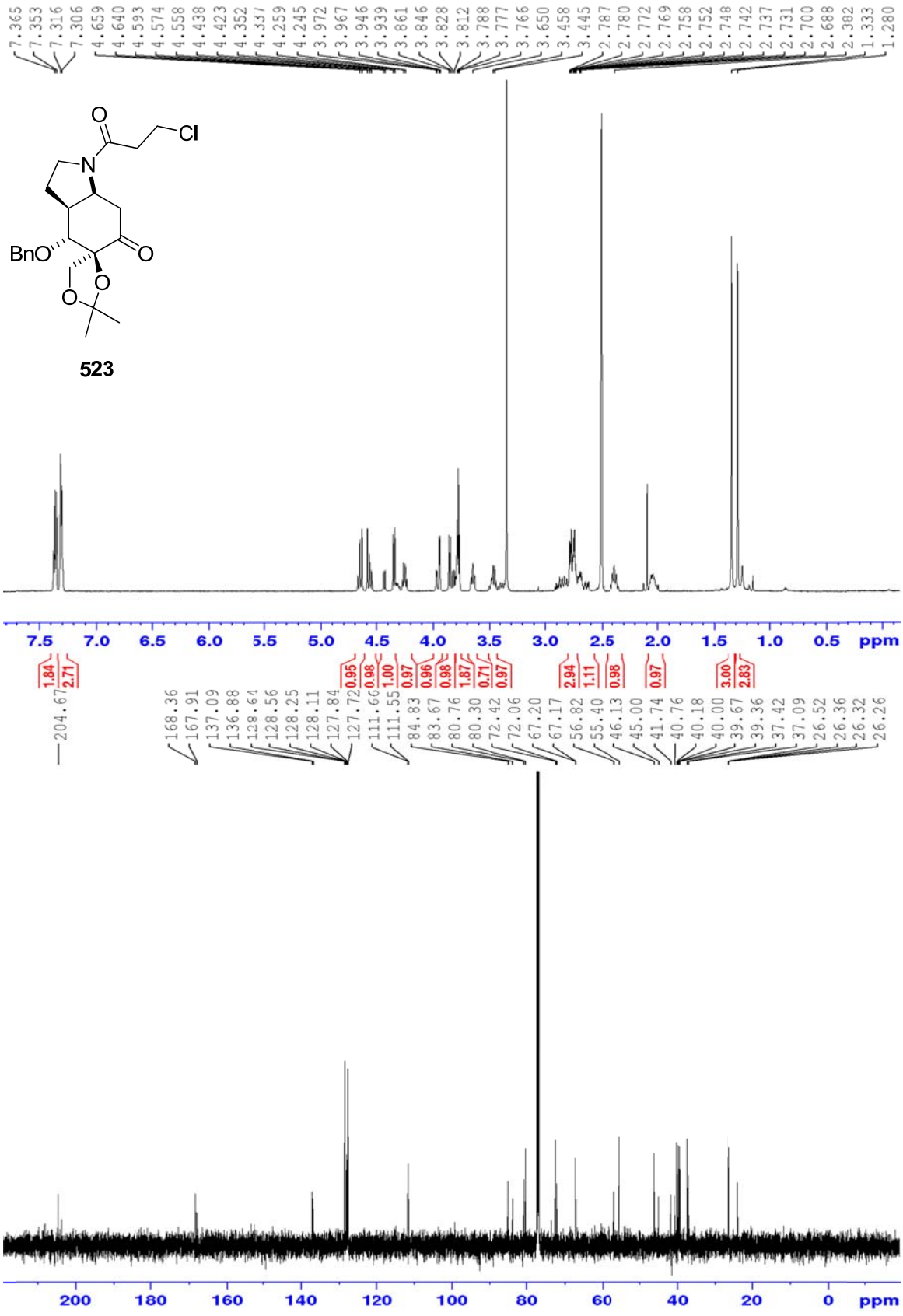
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7.0 References

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8.0 Vita

David Ryan Adams was born on Friday, July 13, 1979 in Albany, Georgia to the parents of Bill (RIP) and Margot Adams. He and his four siblings, Kelli, Michael, Marc, and Bret, were raised by their parents in both North Carolina and Florida. While in Florida he attended the University of Florida in Gainesville. While an undergraduate in Gainesville he studied synthetic chemistry under Professor Tomas Hudlicky and worked in his laboratory under the direct tutelage of Dr. Uwe Rinner. Upon graduation in 2007, David was married to his long time love Candice Weaver in Ormond Beach, Florida and was subsequently employed by Albany Molecular Research, Inc. in Albany, NY as a synthetic chemist. After approximately two years of employment he moved to St. Catharines, Ontario to pursue graduate studies with Professor Tomas Hudlicky. Under the mentorship of Professor Hudlicky David has become a lifelong student of synthetic organic chemistry and in his time at Brock University has pursued the synthesis of natural products.

Also while in Ontario David and Candice became the proud parents of two beautiful daughters, Emma and Hannah Adams, for whom everything henceforth has been worked for.